WEST Search History

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DATE: Monday, January 31, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	pky2 or pky-2	6
☐	L2	tyrosine near5 kinase	17429
☐	L3	L2 near5 (proline or prolinerich or proline-rich or raftk or cak or cadtk or cad or ca or raf)	279
☐	L4	inhibit$	944332
☐	L5	modulat$	689369
☐	L6	antagon$	118718
☐	L7	block$	3270272
☐	L8	inactivat$	116992
☐	L9	enhanc$	1602803
☐	L10	compet$	220467
☐	L11	(L10 or l9 or l8 or l7 or l6 or l5 or l4).clm.	527355
☐	L12	l3.clm.	6
☐	L13	L12 not l1	6
☐	L14	signal near5 transduct$	27543
☐	L15	signal near5 transduc$	125383
☐	L16	L15.clm.	21637
☐	L17	L16 and l11	5119
☐	L18	L17 and (raf or cad or ca or raftk or cak or cadtk or pyk or pyk2 or pyk-2 or kinase).clm.	310
☐	L19	L18 and (method or process or screen or screening or detect or identifying or indentify).clm.	301
☐	L20	tyrphostins or quinazolines or quinoxolines or quinolines or indolinones or tyr-phostins or quin-azolines or quin-oxo-lines or quin-oxolines or quin-olines or indo-linones	65853
☐	L21	L20 and l19	67
☐	L22	('5804396' |'6689806' |'5763198')!.PN.	6

END OF SEARCH HISTORY

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Search Results - Record(s) 51 through 67 of 67 returned.

☐ 51. 6225335. 15 Dec 98; 01 May 01. 3-(4'-bromobenzylindenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/404 C07D209/34.

☐ 52. 6204267. 01 May 98; 20 Mar 01. Methods of modulating serine/thereonine protein kinase function with quinazoline-based compounds. Tang; Peng C., et al. 514/252.17; 514/266.2 514/266.21 514/266.4 544/284 544/285 544/286 544/287 544/288 544/289 544/291 544/292 544/293. A61K031/495 A61K031/505 C07D239/72.

☐ 53. 6187756. 28 Jan 00; 13 Feb 01. Composition and methods for treatment of neurological disorders and neurodegenerative diseases. Lee; Robert K. K., et al. 514/26; 514/169 514/182 514/573 514/878 514/879. A61K031/70.

☐ 54. 6147106. 20 Aug 97; 14 Nov 00. Indolinone combinatorial libraries and related products and methods for the treatment of disease. Tang; Peng Cho, et al. 514/414; 514/235.2 514/323 544/143 544/144 546/201 548/455 548/460. A61K031/404 C07D403/06.

☐ 55. 6043224. 05 Sep 97; 28 Mar 00. Compositions and methods for treatment of neurological disorders and neurodegenerative diseases. Lee; Robert K. K., et al. 514/26; 514/169 514/182 514/573. A61K003/705.

☐ 56. 5962265. 15 Dec 98; 05 Oct 99. Human signal transduction serine/threonine kinase. Norris; Tyrrell Errick, et al. 435/69.1; 435/320.1 435/325 435/375 435/70.1 514/44 536/23.1 536/24.3 536/24.5. C12N015/00 C12N015/63 C07H021/04 A61K048/00.

☐ 57. 5908839. 23 Aug 96; 01 Jun 99. Asthma associated factors as targets for treating atopic allergies including asthma and related disorders. Levitt; Roy Clifford, et al. 514/182; 514/826. A61K031/56.

☐ 58. 5886020. 05 Jun 96; 23 Mar 99. 3-(4'-dimethylaminobenzylidenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/40 C07D209/34.

☐ 59. 5883116. 05 Jun 96; 16 Mar 99. 3-(2'-alkoxybenzylidenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/40 C07D209/34.

☐ 60. 5883113. 05 Jun 96; 16 Mar 99. 3-(4'-Bromobenzylindenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/40 C07D209/34.

☐ 61. 5880141. 07 Jun 95; 09 Mar 99. Benzylidene-Z-indoline compounds for the treatment of disease. Tang; Peng Cho, et al. 514/339; 514/414 514/418 546/277.4 548/468 548/486. A61K031/405 C07D209/34.

☐ 62. 5858981. 07 Jun 96; 12 Jan 99. Method of inhibiting phagocytosis. Schreiber; Alan D., et al. 514/18; 514/2 514/44 530/300 530/330 536/24.5. A61K038/00 A61K038/07 A01N043/34 C07H021/04.

☐ 63. 5834504. 05 Jun 96; 10 Nov 98. 3-(2'-halobenzylidenyl)-2-indolinone compounds for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. H61K031/40 C07D209/34.

☐ 64. 5804396. 12 Oct 94; 08 Sep 98. Assay for agents active in proliferative disorders. Plowman; Gregory D.. 435/7.23; 435/7.2 436/63 436/64. G01N033/574 G01N033/53 G01N033/567 G01N033/48.

☐ 65. 5792783. 05 Jun 96; 11 Aug 98. 3-heteroaryl-2-indolinone compounds for the treatment of disease. Tang; Peng Cho, et al. 514/397; 514/359 514/361 514/362 514/363 514/364 514/365 514/372 514/374 514/378 514/381 514/383 514/406 514/414 548/125 548/127 548/128 548/131 548/134 548/136 548/143 548/202 548/214 548/235 548/247 548/250 548/255 548/266.4 548/312.1 548/364.7 548/468. A61K031/40 A61K031/415 C07D403/06.

☐ 66. 5763198. 22 Jul 94; 09 Jun 98. Screening assays for compounds. Hirth; Klaus Peter, et al. 435/7.21; 435/15 435/21 435/29 435/7.23 435/7.24 435/7.94 436/518 436/548. G01N033/543.

☐ 67. 5721266. 26 Sep 94; 24 Feb 98. Substituted imidazolinyl-imidazolines as antagonists of SH-2 binding and therapeutic uses thereof. Decker; Stuart James, et al. 514/398; 514/400 514/401 514/402. A01N043/50.

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Search Results - Record(s) 1 through 6 of 6 returned.

L22: Entry 1 of 6 File: USPT Feb 10, 2004

US-PAT-NO: 6689806
DOCUMENT-IDENTIFIER: US 6689806 B1

TITLE: Indolinone compounds as kinase inhibitors

DATE-ISSUED: February 10, 2004

US-CL-CURRENT: 514/418; 514/414, 514/415, 548/465, 548/466, 548/468, 548/486

INT-CL: [07] A61 K 31/404, C07 D 209/34

L22: Entry 2 of 6 File: USPT Sep 8, 1998

US-PAT-NO: 5804396
DOCUMENT-IDENTIFIER: US 5804396 A

TITLE: Assay for agents active in proliferative disorders

DATE-ISSUED: September 8, 1998

US-CL-CURRENT: 435/7.23; 435/7.2, 436/63, 436/64

INT-CL: [06] G01 N 33/574, G01 N 33/53, G01 N 33/567, G01 N 33/48

L22: Entry 3 of 6 File: USPT Jun 9, 1998

US-PAT-NO: 5763198
DOCUMENT-IDENTIFIER: US 5763198 A

TITLE: Screening assays for compounds

DATE-ISSUED: June 9, 1998

US-CL-CURRENT: 435/7.21; 435/15, 435/21, 435/29, 435/7.23, 435/7.24, 435/7.94, 436/518, 436/548

INT-CL: [06] G01 N 33/543

L22: Entry 4 of 6 File: DWPI Feb 10, 2004

DERWENT-ACC-NO: 2000-628241
ABSTRACTED-PUB-NO: WO 200056709A

TITLE: New indolinone derivatives are protein kinase inhibitors used for treating e.g. cardiovascular, dermatological and immune disorders and cancer

INT-CL (IPC): A61 K 31/40, A61 K 31/404, A61 K 31/4439, A61 K 31/5377, A61 P 3/10,

A61 P 9/00, A61 P 9/10, A61 P 17/06, A61 P 19/02, A61 P 29/00, A61 P 35/00, A61 P 37/00, A61 P 43/00, C07 D 209/34, C07 D 209:00, C07 D 401/04 , C07 D 401/06, C07 D 401/10, C07 D 401/12, C07 D 401/14, C07 D 403/06, C07 D 403/10, C07 D 409/04, C07 D 409/10, C07 D 409/14, C07 D 413/12, C07 D 413/14, C12 N 9/99, C07 D 213:00, C07 D 401/12, C07 D 213:00, C07 D 401/06, C07 D 333:00, C07 D 409/10, C07 D 409/10, C07 D 401/12, C07 D 401/06, C07 D 333:00, C07 D 213:00, C07 D 213:00, C07 D 209:00, C07 D 209:00, C07 D 209:00
Derwent-CL (DC): B02
CPI Codes: B06-D01; B11-C08E1; B12-K04E; B14-C03; B14-C09; B14-D06; B14-F01; B14-F02; B14-F07; B14-G03; B14-H01; B14-N17C; B14-S04;

L22: Entry 5 of 6 File: DWPI Sep 8, 1998

DERWENT-ACC-NO: 1998-505647
ABSTRACTED-PUB-NO: US 5804396A

TITLE: Screening assays for anticancer drugs - based on inhibition of HER tyrosine kinase activity

INT-CL (IPC): G01 N 33/48, G01 N 33/53, G01 N 33/567, G01 N 33/574
Derwent-CL (DC): B04, D16 , S03
CPI Codes: B04-L04; B04-M01; B11-C08E3; B12-K04A1; D05-A01B2; D05-H09; D05-H12D; D05-H14B;
EPI Codes: S03-E14H; S03-E14H4;

L22: Entry 6 of 6 File: DWPI Jun 9, 1998

DERWENT-ACC-NO: 1998-347315
ABSTRACTED-PUB-NO: US 5763198A

TITLE: Screening assays for phosphotyrosine modulators - to identify compounds to treat diseases in which tyrosine kinase or phosphatase activity in signal transduction pathway is important

INT-CL (IPC): G01 N 33/543
Derwent-CL (DC): B04, D16 , J04 , S03
CPI Codes: B04-G03; B04-L04; B11-C07A2; B11-C07A3; B11-C07A4; B11-C07A5; B12-K04; D05-H09; J04-C03;
EPI Codes: S03-E14H4;

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Search Results - Record(s) 51 through 67 of 67 returned.

☐ 51. 6225335. 15 Dec 98; 01 May 01. 3-(4'-bromobenzylindenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/404 C07D209/34.

☐ 52. 6204267. 01 May 98; 20 Mar 01. Methods of modulating serine/thereonine protein kinase function with quinazoline-based compounds. Tang; Peng C., et al. 514/252.17; 514/266.2 514/266.21 514/266.4 544/284 544/285 544/286 544/287 544/288 544/289 544/291 544/292 544/293. A61K031/495 A61K031/505 C07D239/72.

☐ 53. 6187756. 28 Jan 00; 13 Feb 01. Composition and methods for treatment of neurological disorders and neurodegenerative diseases. Lee; Robert K. K., et al. 514/26; 514/169 514/182 514/573 514/878 514/879. A61K031/70.

☐ 54. 6147106. 20 Aug 97; 14 Nov 00. Indolinone combinatorial libraries and related products and methods for the treatment of disease. Tang; Peng Cho, et al. 514/414; 514/235.2 514/323 544/143 544/144 546/201 548/455 548/460. A61K031/404 C07D403/06.

☐ 55. 6043224. 05 Sep 97; 28 Mar 00. Compositions and methods for treatment of neurological disorders and neurodegenerative diseases. Lee; Robert K. K., et al. 514/26; 514/169 514/182 514/573. A61K003/705.

☐ 56. 5962265. 15 Dec 98; 05 Oct 99. Human signal transduction serine/threonine kinase. Norris; Tyrrell Errick, et al. 435/69.1; 435/320.1 435/325 435/375 435/70.1 514/44 536/23.1 536/24.3 536/24.5. C12N015/00 C12N015/63 C07H021/04 A61K048/00.

☐ 57. 5908839. 23 Aug 96; 01 Jun 99. Asthma associated factors as targets for treating atopic allergies including asthma and related disorders. Levitt; Roy Clifford, et al. 514/182; 514/826. A61K031/56.

☐ 58. 5886020. 05 Jun 96; 23 Mar 99. 3-(4'-dimethylaminobenzylidenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/40 C07D209/34.

☐ 59. 5883116. 05 Jun 96; 16 Mar 99. 3-(2'-alkoxybenzylidenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/40 C07D209/34.

☐ 60. 5883113. 05 Jun 96; 16 Mar 99. 3-(4'-Bromobenzylindenyl)-2-indolinone and analogues thereof for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. A61K031/40 C07D209/34.

☐ 61. 5880141. 07 Jun 95; 09 Mar 99. Benzylidene-Z-indoline compounds for the treatment of disease. Tang; Peng Cho, et al. 514/339; 514/414 514/418 546/277.4 548/468 548/486. A61K031/405 C07D209/34.

☐ 62. 5858981. 07 Jun 96; 12 Jan 99. Method of inhibiting phagocytosis. Schreiber; Alan D., et al. 514/18; 514/2 514/44 530/300 530/330 536/24.5. A61K038/00 A61K038/07 A01N043/34 C07H021/04.

☐ 63. 5834504. 05 Jun 96; 10 Nov 98. 3-(2'-halobenzylidenyl)-2-indolinone compounds for the treatment of disease. Tang; Peng Cho, et al. 514/418; 548/486. H61K031/40 C07D209/34.

☐ 64. 5804396. 12 Oct 94; 08 Sep 98. Assay for agents active in proliferative disorders. Plowman; Gregory D.. 435/7.23; 435/7.2 436/63 436/64. G01N033/574 G01N033/53 G01N033/567 G01N033/48.

☐ 65. 5792783. 05 Jun 96; 11 Aug 98. 3-heteroaryl-2-indolinone compounds for the treatment of disease. Tang; Peng Cho, et al. 514/397; 514/359 514/361 514/362 514/363 514/364 514/365 514/372 514/374 514/378 514/381 514/383 514/406 514/414 548/125 548/127 548/128 548/131 548/134 548/136 548/143 548/202 548/214 548/235 548/247 548/250 548/255 548/266.4 548/312.1 548/364.7 548/468. A61K031/40 A61K031/415 C07D403/06.

☐ 66. 5763198. 22 Jul 94; 09 Jun 98. Screening assays for compounds. Hirth; Klaus Peter, et al. 435/7.21; 435/15 435/21 435/29 435/7.23 435/7.24 435/7.94 436/518 436/548. G01N033/543.

☐ 67. 5721266. 26 Sep 94; 24 Feb 98. Substituted imidazolinyl-imidazolines as antagonists of SH-2 binding and therapeutic uses thereof. Decker; Stuart James, et al. 514/398; 514/400 514/401 514/402. A01N043/50.

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US006689806B1

(12) **United States Patent**
Tang et al.

(10) **Patent No.: US 6,689,806 B1**
(45) **Date of Patent: Feb. 10, 2004**

(54) **INDOLINONE COMPOUNDS AS KINASE INHIBITORS**

(75) Inventors: **Peng Cho Tang**, Moraga, CA (US); **Li Sun**, Foster City, CA (US); **Gerald McMahon**, San Francisco, CA (US); **Todd Anthony Miller**, Bend, OR (US); **Shahrzad Shirazian**, Corte Madera, CA (US); **Chung Chen Wei**, Foster City, CA (US); **G. Davis Harris, Jr.**, San Francisco, CA (US); **Xiaoyuan Li**, Los Altos, CA (US); **Congxin Liang**, Sunnyvale, CA (US)

(73) Assignee: **Sugen, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/534,405**

(22) Filed: **Mar. 22, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/125,945, filed on Mar. 24, 1999, provisional application No. 60/127,863, filed on Apr. 5, 1999, provisional application No. 60/131,192, filed on Apr. 26, 1999, and provisional application No. 60/132,243, filed on May 3, 1999.

(51) **Int. Cl.**[7] **A61K 31/404**; C07D 209/34

(52) **U.S. Cl.** **514/418**; 514/414; 514/415; 548/468; 548/465; 548/466; 548/486

(58) **Field of Search** 548/468, 465, 548/466, 486; 514/414, 415, 418

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,308,134	A		3/1967	Plostnieks
4,002,749	A		1/1977	Rovnyak et al. 424/246
4,053,613	A		10/1977	Rovnyak et al. 424/246
4,966,849	A		10/1990	Vallee et al. 435/199
5,206,261	A	*	4/1993	Kawaguchi et al. 514/418
5,217,999	A		6/1993	Levitzki et al. 514/613
5,302,606	A		4/1994	Spada et al. 514/357
5,322,950	A	*	6/1994	Sircar et al. 548/253
5,330,992	A		7/1994	Elssenstat et al. 514/312
5,786,488	A		7/1998	Tang et al. 548/455
5,792,783	A	*	8/1998	Tang et al. 514/397
5,840,745	A		11/1998	Buzzetti et al. 514/414
5,880,141	A		3/1999	Tang et al. 514/339
5,883,113	A		3/1999	Tang et al. 514/418
5,883,116	A		3/1999	Tang et al. 514/418
5,886,020	A		3/1999	Tang et al. 514/418
RE36,256	E		7/1999	Spada et al. 514/249

FOREIGN PATENT DOCUMENTS

EP	0 252 731 A2		1/1988
EP	0 304 493 B1		3/1989
EP	0 586 226 A1		10/1993
FR	1 599 772 A		7/1970
GB	1290686	*	9/1972
WO	91-13055		9/1991
WO	91-15495		10/1991
WO	92-07830		5/1992
WO	92-20642		11/1992
WO	92-21660		12/1992
WO	WO 93/01182 A		1/1993
WO	93-23040		11/1993
WO	94-03427		2/1994
WO	94-10202		5/1994
WO	94-14808		7/1994
WO	WO 95/01349 A		1/1995
WO	95-14667		6/1995
WO	95-24190		9/1995
WO	96-00226		1/1996
WO	96-16964		6/1996
WO	WO 96/22976 A		8/1996
WO	96-22976		8/1996
WO	WO 96/32380 A		10/1996
WO	96-40116		12/1996
WO	98-07695		2/1998
WO	98-07835		2/1998
WO	WO 98/24432 A		6/1998
WO	98-45708		10/1998
WO	98-50356		11/1998
WO	98-56376		12/1998
WO	99/10325		3/1999
WO	99/19325		4/1999
WO	WO 9961422	*	12/1999

OTHER PUBLICATIONS

B.Franco et al,"Cinnamamide analogues as inhib.or PTK", Farmaco, 48/5,615–36(1993).*

Tao et al,"Electrochem. & peroxid. O2 mediated";J. Electroanal. Chem. 432/1–2,7–18(1997).*

A.Aldo et al,"Synth. & Cardiotonic Activity of indolinone", Eur.J.Med.Chem.,25/2,187–90(1990).*

E.Ali et al,"Regiosel.Pd–(II)–Cataly. Synthe.";J.Am.Chem. Soc. 118/18,4264–70(1996).*

Akbasak and Sunar–Akbasak., "Oncogenes: cause or consequence in the development of glial tumors," *J. Neurol. Sci.* 111:119–133 (1992).

Andreani et al., "Synthesis and potential coanthracyclinic activity of substituted 3–(5–imidazo[2,1–b] thiazolylmethylene)–2–indolinones," *Eur. J. Med. Chem.* 32:919–924 (1997).

Arteaga et al., "Blockade of the Type I Somatomedin Receptor Inhibits Growth of Human Breast Cancer Cells in Athymic Mice," *J. Clin. Invest.* 84:1418–1423 (1989).

(List continued on next page.)

Primary Examiner—Mukund J. Shah
Assistant Examiner—Sudhaker B. Patel
(74) *Attorney, Agent, or Firm*—Beth A. Burrous; Foley & Lardner

(57) **ABSTRACT**

The invention relates to certain indolinone compounds, their method of synthesis, and a combinatorial library consisting of the indolinone compounds of the invention. The invention also relates to methods of modulating the function of protein kinases using indolinone compounds of the invention and methods of treating diseases by modulating the function of protein kinases and related signal transduction pathways.

19 Claims, No Drawings

First Hit

L21: Entry 1 of 67 File: PGPB Jan 27, 2005

PGPUB-DOCUMENT-NUMBER: 20050020516
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050020516 A1

TITLE: Effects of combined administration of farnesyl transferase inhibitors and signal transduction inhibitors

PUBLICATION-DATE: January 27, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Daley, George Q.	Weston	MA	US	
Hoover, Russell R.	Brighton	MA	US	

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	COUNTRY	TYPE CODE
Whitehead Institute for Biomedical Research	Cambridge	MA		02

APPL-NO: 10/ 870403 [PALM]
DATE FILED: June 17, 2004

RELATED-US-APPL-DATA:
Application 10/870403 is a continuation-of US application 09/971365, filed October 4, 2001, ABANDONED
Application is a non-provisional-of-provisional application 60/238240, filed October 5, 2000,
Application is a non-provisional-of-provisional application 60/238813, filed October 6, 2000,

INT-CL: [07] A61 K 31/704, A61 K 31/33

US-CL-PUBLISHED: 514/035; 514/183
US-CL-CURRENT: 514/35; 514/183

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention relates to methods of reducing proliferation of cells, enhancing apoptosis of cells or both in an individual in need thereof, comprising administering to the individual a combination of at least one farnesyl transferase inhibitor (FTI), such as an inhibitor of Ras function, and at least one signal transduction inhibitor (STI) in a therapeutically effective amount, wherein proliferation of cells is reduced and/or apoptosis of cells is enhanced in the individual. In one embodiment, the invention relates to a method of reducing proliferation of STI resistant cells, enhancing apoptosis of STI resistant cells, or both in an individual in need thereof, comprising administering to the individual a combination of at least one FTI and at least one STI in a

therapeutically effective amount, wherein proliferation of STI resistant cells is reduced and/or apoptosis of STI resistant cells is enhanced in the individual. The present invention can be used to treat leukemia (e.g., CML) in an individual comprising administering to the individual a combination of at least one FTI and at least one STI in a therapeutically effective amount.

RELATED APPLICATION(S)

[0001] This application is a continuation of U.S. application Ser. No. 09/971,365, filed Oct. 4, 2001, which claims the benefit of U.S. Provisional Application No. 60/238,240, filed on Oct. 5, 2000 and claims the benefit of U.S. Provisional Application No. 60/238,813, filed on Oct. 6, 2000. The entire teachings of the above above applications are incorporated herein by reference.

DOCUMENT-IDENTIFIER: US 20040076607 A1
TITLE: Asthma associated factors as targets for treating atopic allergies including asthma and related disorders

Detail Description Paragraph:
[0108] In addition to the direct regulation of the IL-9 receptor, this invention also encompasses methods of downstream regulation which involve inhibition of signal transduction. In particular, a further embodiment of this invention is drawn to inhibition of tyrosine phosphorylation. It is known in the art that highly exergonic phosphoryl-transfer reactions are catalyzed by various enzymes known as kinases. In other words, a kinase transfers phosphoryl groups between ATP and a metabolite. IL-9 induces tyrosine phosphorylation of multiple proteins; it is known in the art that in addition to the activation of JAK1 and JAK3 tyrosine kinases, IL-9 also induces tyrosine phosphorylation of Stat3..sup.58 Phoshorylation of Stat3 is unique to the IL-9 signal transduction pathway and hence is a perfect target for inhibitors..sup.58 This invention includes within its scope tyrphostins which are specific inhibitors of protein tyrosine kinases. Thus, tyrphostins (obtained for example from Calbiochem) and other similar inhibitors of these kinases are useful in the modulation of signal transduction and are useful in the treatment of atopic allergies and asthma.

CLAIMS:

10. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the purified and isolated DNA molecule of claim 1.

11. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the purified and isolated RNA molecule of claim 9.

12. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the purified and isolated DNA molecule of claim 4.

13. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the purified and isolated protein molecules of claim 6.

14. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the chemically synthesized molecules of claim 7.

15. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the recombinant DNA molecule of claim 8.

18. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of a soluble interleukin-9 receptor or an active fragment thereof.

19. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of an antibody specific for

human interleukin-9 or the interleukin-9 receptor that can be administered in an amount sufficient to down regulate the activity of interleukin-9.

34. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the purified and isolated molecule of claim 26.

43. A method of alleviating asthma-related disorders by down-regulating the activity of interleukin-9 by administering to patients in need of such treatment an effective amount of the purified and isolated protein molecule of claim 35.

55. A mammalian cell comprising a DNA sequence encoding an interleukin-9 polypeptide and modified in vitro to permit enhanced expression of the interleukin-9 polypeptide by a homologous recombinational event comprising the step of inserting an expression regulatory sequence in functional proximity to the interleukin-9 polypeptide encoding sequence.

58. A method for preparing an interleukin-9 polypeptide comprising the steps of: (a) culturing the DNA molecule of claim 1 under conditions that provide for expression of the interleukin-9 polypeptide; and (b) recovering the expressed interleukin-9 polypeptide.

62. A method of preparing an antibody specific to an interleukin-9 polypeptide which comprises the DNA molecule of claim 1 comprising the steps of: (a) conjugating an interleukin-9 polypeptide which corresponds to the DNA molecule of claim 1 to a carrier protein; (b) immunizing a host animal with the interleukin-9 polypeptide fragment-carrier protein conjugate of step (a) admixed with an adjuvant; and (c) obtaining antibody from the immunized host animal.

63. A method of quantifying interleukin-9 polypeptide which comprises the DNA molecule of claim 1 comprising the steps of: (a) contacting a sample suspected of containing interleukin-9 polypeptides with an antibody that specifically binds to the interleukin-9 polypeptides under conditions that allow for the formation of reaction complexes comprising the antibody and these interleukin-9 polypeptides; and (b) detecting the formation of reaction complexes comprising the antibody and interleukin-9 polypeptides in the sample, wherein detection of the formation of reaction complexes indicates the presence of interleukin-9 polypeptides in the sample.

64. The method of claim 63, wherein said antibody is bound to a solid phase support.

65. An in vitro method for evaluating the levels of interleukin-9 polypeptides containing methionine at position 117 or fragments thereof in a biologic sample comprising the steps of: (a) detecting the formation of reaction complexes in a biological sample according to the method of claim 63, and (b) evaluating the amount of reaction complexes formed, which amount of reaction complexes corresponds to the level of interleukin-9 polypeptides in the biological sample.

66. An in vitro method for detecting or diagnosing susceptibility to atopy, asthma, or a related disorder associated with elevated levels of interleukin-9 polypeptide in a human subject comprising the steps of: (a) evaluating the level of interleukin-9 polypeptides in a biological sample; and (b) comparing the level of interleukin-9 polypeptides present in normal subjects or in the subjects at an earlier time, wherein an increase in the level of interleukin-9 polypeptides as compared to normal levels indicates a predisposition to atopy, asthma, and related disorders.

67. The in vitro method of claim 66, wherein the interleukin-9 polypeptide evaluated is Theorine 117.

68. An in vitro method for detecting or diagnosing the presence of atopy, asthma, or related disorders associated with a lack of a methionine at position 117 of the interleukin-9 polypeptide in a human subject comprising: (a) evaluating the amount of a specific interleukin-9 polypeptide in a biological sample; and (b) comparing the level of interleukin-9 polypeptide present in non-atopic subjects or in the subjects at an earlier time, wherein an increase in the level of a specific interleukin-9 polypeptide as compared to non-atopic levels indicates a predisposition to atopy, asthma, and related disorders.

69. The in vitro method of claim 68, wherein the specific interleukin-9 polypeptide evaluated is Theorine 117.

70. An in vitro method for monitoring a therapeutic treatment of atopy, asthma, and related disorders in a mammalian subject comprising evaluating the levels of a specific interleukin-9 polypeptide in a series of biologic samples obtained at different time points from a human subject undergoing therapeutic treatment with polypeptides having the sequence of human interleukin-9 containing methionine at position 117 or fragments thereof.

75. A pharmaceutical composition for decreasing the function of interleukin-9 polypeptide comprising an antagonist of interleukin-9 polypeptide which comprises the chemically synthesized molecule of claim 7 in a pharmaceutically acceptable carrier.

76. A pharmaceutical composition for decreasing the function of interleukin-9 polypeptide comprising an antagonist of interleukin-9 polypeptide which comprises the protein molecule of claim 6 in a pharmaceutically acceptable carrier.

77. A pharmaceutical composition for decreasing the function of interleukin-9 polypeptide comprising an antagonist of interleukin-9 polypeptide which comprises the DNA molecule of claim 1 in a pharmaceutically acceptable carrier.

78. The pharmaceutical composition of claim 75, wherein the antagonist is selected from the group comprising an antibody that binds to the interleukin-9 polypeptide and an antibody that binds to the interleukin-9 receptor.

79. The pharmaceutical composition of claim 76, wherein the antagonist is selected from the group comprising an antibody that binds to the interleukin-9 polypeptide and an antibody that binds to the interleukin-9 receptor.

80. The pharmaceutical composition of claim 77, wherein the antagonist is selected from the group comprising an antibody that binds to the interleukin-9 polypeptide and an antibody that binds to the interleukin-9 receptor.

81. A method of alleviating asthma-related disorders by administering to patents in need of such a treatment a compound that will down regulate the function of either IL-9 or the IL-9 receptor.

82. The method of claim 81, wherein said compound is an inhibitor of the signal transduction of protein tyrosine kinase.

83. The method of claim 82, wherein said inhibitor is selected from kinase inhibitors comprising Tyrphostins.

84. The method of claim 81, wherein said compound is selected from a group of aminosterols described in FIG. 28.

85. The method of claim 81, wherein the compound inhibits the interaction of IL-9 with the IL-9 receptor.

86. The method of claim 81, wherein the compound is a substitution or deletion analogue or fragment of human IL-9.

87. The method of claim 86, wherein the compound is sequence ID NO: 15(KP-23).

88. The method of claim 86, wherein the compound is sequence ID No: 16(KP-24).

89. The method of claim 85, wherein the compound is human IL-9 containing a Met residue at position 117 or a fragment thereof.

90. The method of claim 89, wherein the compound is sequence ID NO: 13(KP-16).

91. The method of claim 89, wherein the compound is sequence ID NO: 14(KP-20).

100. A composition comprising an IL-9 antagonist selected from the group consisting of peptides having the sequence Ser-Asp-Asn-Ala-Thr-Arg-Pro-Al- a-Phe-Ser-Glu-Arg-Leu-Ser-Gln-Met-Thr-Asn (Seq. ID No. 13); Phe-Ser-Arg-Val-Lys-Lys-Ser-Val-Glu-Val-Leu-Lys-Asn-Asn-Lys-Ala-Pro-Tyr (Seq. ID No. 14); and Glu-Gln-Pro-Ala-Asn-Gln-Thr-Thr-Ala-Gly-Asn-Ala-Leu- -Thr-Phe-Leu-Lys-Ser (Seq. ID No. 15).

101. The method of claim 81 wherein the compound has a configuration substantially similar to the 3-D structure corresponding to amino acids 44-89 of human IL-9 or a fragment thereof.

102. A segment of human IL-9 that elicits antibodies that block the binding of human IL-9 to its receptor.

104. A segment of the human IL-9 receptor that elicits antibodies that block the binding of human IL-9 to the receptor.

106. An epitope from human IL-9 that binds to compounds that block the binding of IL-9 to its receptor.

108. A method for identifying antagonists of IL-9 or the IL-9 receptor comprising the steps of: a) obtaining animals susceptible to airway hyperresponsiveness; b) administering antigens that induce airway hyperresponsivness; c) comparing the characteristics of any resulting airway hyperresponsiveness with the characteristics of airway hyperresponsiveness obtained with pretreatment with a possible IL-9 or IL-9 receptor antagonist agent; and d) selecting those agents for which pretreatment diminished the characteristics.

109. The method of claim 9 wherein the animal expresses the human IL-9 receptor.

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(54) ASTHMA ASSOCIATED FACTORS AS TARGETS FOR TREATING ATOPIC ALLERGIES INCLUDING ASTHMA AND RELATED DISORDERS

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(57) ABSTRACT

A C to T DNA variation at position 3365 in exon 5 of the human Asthma Associated Factor 1 (AAF1) produces the predicted amino acid substitution of a methionine for a threonine at codon 117 of AAF1. When this substitution occurs in both alleles in one individual, it is associated with less evidence of atopic allergy including asthma, fewer abnormal skin test responses, and a lower serum total IgE. Thus, applicant has identified the existence of a non-asthmatic, non-atopic phenotype characterized by methionine at codon 117 when it occurs in both AAF1 gene products in one individual.

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TITLE: Methods of modulating protein tyrosine kinase function with substituted indolinone compounds

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Application 09/129256 is a continuation-in-part-of US application 08/915366, filed August 20, 1997, US Patent No. 6147106

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US-CL-CURRENT: 435/7.1; 514/291, 514/411, 546/81, 548/427, 548/429

ABSTRACT:

The invention relates to certain indolinone compounds, their method of synthesis, and a combinatorial library consisting of the indolinone compounds. The invention also relates to methods of modulating the function of protein tyrosine kinases using indolinone compounds and methods of treating diseases by modulating the function of protein tyrosine kinases and related signal transduction pathways.

RELATED APPLICATIONS

[0001] This application is related to the U.S. patent application Ser. No. 08/915,366, filed Aug. 8, 1997, by Tang et al., and entitled "INDOLINONE COMBINATORIAL LIBARIES AND RELATED PRODUCTS AND METHODS FOR THE TREATMENT OF DISEASE" (Lyon & Lyon Docket No. 227/111), which is hereby incorporated by reference herein in its entirety, including any drawings.

DOCUMENT-IDENTIFIER: US 20040005648 A1
TITLE: PYK2 related products and methods

Summary of Invention Paragraph:
[0049] In preferred embodiments, the compound inhibits a phosphorylation activity of PYK2 and is selected from the group consisting of tyrphostins, quinazolines, quinaxolines, and quinolines. The present invention also features compounds capable of binding and inhibiting PYK2 polypeptide that are identified by methods described above.

Summary of Invention Paragraph:
[0062] In preferred embodiments the screening method involves growing cells (i.e., in a dish) that either naturally or recombinantly express a G-coupled protein receptor, PYK2, and RAK. The test compound is added at a concentration from 0.1 uM to 100 uM and the mixture is incubated from 5 minutes to 2 hours. The ligand is added to the G-coupled protein receptor for preferably 5 to 30 minutes and the cells are lysed. RAK is isolated using immunoprecipitation or ELISA by binding to a specific monoclonal antibody. The amount of phosphorylation compared to cells that were not exposed to a test compound is measured using an anti-phosphotyrosine antibody (preferably polyclonal). Examples of compounds that could be tested in such screening methods include tyrphostins, quinazolines, quinoxolines, and quinolines.

Summary of Invention Paragraph:
[0063] The quinazolines, tyrphostins, quinolines, and quinoxolines referred to above include well known compounds such as those described in the literature. For example, representative publications describing quinazoline include Barker et al., EPO Publication No. 0 520 722 A1; Jones et al., U.S. Pat. No. 4,447,608; Kabbe et al., U.S. Pat. No. 4,757,072; Kaul and Vougioukas, U.S. Pat. No. 5, 316,553; Kreighbaum and Corner, U.S. Pat. No. 4,343,940; Pegg and Wardleworth, EPO Publication No. 0 562 734 Al; Barker et al., Proc. of Am. Assoc. for Cancer Research 32:327 (1991); Bertino, J. R., Cancer Research 3:293-304 (1979); Bertino, J. R., Cancer Research 9(2 part 1):293-304 (1979); Curtin et al., Br. J. Cancer 53:361-368 (1986); Fernandes et al., Cancer Research 43:1117-1123 (1983); Ferris et al. J. Org. Chem. 44(2):173-178; Fry et al., Science 265:1093-1095 (1994); Jackman et al., Cancer Research 51:5579-5586 (1981); Jones et al. J. Med. Chem. 29(6):1114-1118; Lee and Skibo, Biochemistry 26 (0.23):7355-7362 (1987); Lemus et al., J. Org. Chem. 54:3511-3518 (1989); Ley and Seng, Synthesis 1975:415-522 (1975); Maxwell et al., Magnetic Resonance in Medicine 17:189-196 (1991); Mini et al., Cancer Research 45:325-330 (1985); Phillips and Castle, J. Heterocyclic Chem. 17(19):1489-1596 (1980); Reece et al., Cancer Research 47(11):2996-2999 (1977); Sculier et al., Cancer Immunol. and Immunother. 23:A65 (1986); Sikora et al., Cancer Letters 23:289-295 (1984); Sikora et al., Analytical Biochem. 172:344-355 (1988); all of which are incorporated herein by reference in their entirety, including any drawings.

Summary of Invention Paragraph:
[0065] Quinolines are described in Dolle et al., J. Med. Chem. 37:2627-2629 (1994); MaGuire, J. Med. Chem. 37:2129-2131 (1994); Burke et al., J. Med. Chem. 36:425-432 (1993); and Burke et al. BioOrganic Med. Chem. Letters 2:1771-1774 (1992), all of which are incorporated by reference in their entirety, including any drawings.

Summary of Invention Paragraph:
[0066] Tyrphostins are described in Allen et al., Clin. Exp. Immunol. 91:141-156 (1993); Anafi et al., Blood 82:12:3524-3529 (1993); Baker et al., J. Cell Sci. 102:543-555 (1992); Bilder et al., Amer. Physiol. Soc. pp. 6363-6143:C721-C730 (1991); Brunton et al., Proceedings of Amer. Assoc. Cancer Rsch. 33:558 (1992); Bryckaert et al., Experimental Cell Research 199:255-261 (1992); Dong et al., J.

Leukocyte Biology 53:53-60 (1993); Dong et al., J. Immunol. 151(5):2717-2724 (1993); Gazit et al., J. Med. Chem. 32:2344-2352 (1989); Gazit et al., "J. Med. Chem. 36:3556-3564 (1993); Kaur et al., Anti-Cancer Drugs 5:213-222 (1994); Kaur et al., King et al., Biochem. J. 275:413-418 (1991); Kuo et al., Cancer Letters 74:197-202 (1993); Levitzki, A., The FASEB J. 6:3275-3282 (1992); Lyall et al., J. Biol. Chem. 264:14503-14509 (1989); Peterson et al., The Prostate 22:335-345 (1993); Pillemer et al., Int. J. Cancer 50:80-85 (1992); Posner et al., Molecular Pharmacology 45:673-683 (1993); Rendu et al., Biol. Pharmacology 44(5):881-888 (1992); Sauro and Thomas, Life Sciences 53:371-376 (1993); Sauro and Thomas, J. Pharm. and Experimental Therapeutics 267(3):119-1125 (1993); Wolbring et al., J. Biol. Chem. 269(36):22470-22472 (1994); and Yoneda et al., Cancer Research 51:4430-4435 (1991); all of which are incorporated herein by reference in their entirety, including any drawings.

CLAIMS:

1. Isolated, purified, or enriched nucleic acid encoding a nucleic acid encoding PYK2 polypeptide.

2. A nucleic acid probe for the detection of nucleic acid encoding a PYK2 polypeptide in a sample.

3. Recombinant nucleic acid encoding a PYK2 polypeptide and a vector or a promoter effective to initiate transcription in a host cell.

4. Recombinant nucleic acid comprising a transcriptional region functional in a cell, a sequence complimentary to an RNA sequence encoding a PYK2 polypeptide and a transcriptional termination region functional in a cell.

5. An isolated, purified, recombinant, or enriched PYK2 polypeptide having a phosphorylation activity.

6. A purified antibody having specific binding affinity to a PYK2 polypeptide.

7. A hybridoma which produces an antibody having specific binding affinity to a PYK2 polypeptide.

8. A method of detecting a compound capable of binding to a PYK2 polypeptide comprising the steps of incubating said compound with said PYK2 polypeptide and detecting the presence of said compound bound to said PYK2 polypeptide.

9. The method of claim 8 wherein said compound inhibits a phosphorylation activity of said PYK2 polypeptide and is selected from the group consisting of tyrphostins, quinazolines, quinoxolines, and quinolines.

10. A compound capable of inhibiting the phosphorylation activity of a PYK2 polypeptide identified by the method of claim 9.

11. A method of screening potential agents useful for treatment of a disease or condition characterized by an abnormality in a signal transduction pathway, wherein said signal transduction pathway includes an interaction between a PYK2 polypeptide and a natural binding partner, comprising the step of assaying said potential agents for those able to promote or disrupt said interaction as an indication of a useful said agent.

12. A method for diagnosis of a disease or condition characterized by an abnormality in a signal transduction pathway, wherein said signal transduction pathway includes an interaction between a PYK2 polypeptide and a natural binding partner, comprising the step of detecting the level of said interaction as an indication of said disease or condition.

13. A method for treatment of an organism having a disease or condition characterized by an abnormality in a signal transduction pathway, wherein said signal transduction pathway includes an interaction between a PYK2 polypeptide and a natural binding partner comprising the step of promoting or disrupting said interaction.

14. The method of claim 13 wherein said disease or condition is selected from the group consisting of epilepsy, schizophrenia, extreme hyperactivity in children, chronic pain and acute pain.

15. The method of claim 13 wherein said disease or condition is selected from the group consisting of stroke, alzheimer's disease, parkinson's disease, neurodegenerative diseases, and migraine.

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☐ 1. Document ID: US 6808877 B2

L25: Entry 1 of 33 File: USPT Oct 26, 2004

DOCUMENT-IDENTIFIER: US 6808877 B2
TITLE: Ligands for FPR class receptors that induce a host immune response to a pathogen or inhibit HIV infection

CLAIMS:

1. A method of identifying a binding partner for a formyl peptide receptor (FPR) class receptor that desensitizes an HIV co-receptor comprising the steps of: providing a cell having a CCR5 or CXCR4 receptor and an FPR class receptor; contacting said cell with a candidate binding partner; and identifying the candidate binding partner as a binding partner if the candidate binding partner binds to an FPR class receptor and induces the phosphorylation of the CCR5 or CXCR4 receptor, wherein phosphorylation identifies desensitization.

2. A method of identifying a binding partner for a formyl peptide receptor (FPR) class receptor that desensitizes an HIV co-receptor comprising the steps of: providing a cell having a CCR5 or CXCR4 receptor and an FPR class receptor; contacting said cell with a candidate binding partner; and identifying the candidate binding partner as a binding partner if the candidate binding partner binds to an FPR class receptor and induces the downregulation of the CCR5 or CXCR4 receptor, at the cell surface, wherein downregulation identifies desensitization.

3. A method of identifying a binding partner for a formyl peptide receptor (FPR) class receptor that desensitizes an HIV co-receptor comprising the steps of: providing a cell having a CCR5 or CXCR4 receptor and an FPR class receptor; contacting said cell with a candidate binding partner; and identifying the candidate binding partner as a binding partner if the candidate binding partner binds to an FPR class receptor and inhibits HIV env-mediated fusion, wherein inhibition identifies desensitization.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. D

☐ 2. Document ID: US 6800447 B2

L25: Entry 2 of 33 File: USPT Oct 5, 2004

DOCUMENT-IDENTIFIER: US 6800447 B2
TITLE: Methods for identifying compounds which bind the active CCR5 chemokine receptor

CLAIMS:

1. A method of identifying a compound which binds to a polypeptide sequence comprising one of SEQ ID NO: 5, comprising contacting said polypeptide with a candidate compound and detecting binding of said candidate compound to said polypeptide.

2. A method for identifying a compound which specifically binds to the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO: 5, the method comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor; (b) expressing said receptor under conditions permitting specific binding of said compound to said receptor; (c) exposing said cell to said compound; and (d) detecting the presence of said compound which has specifically bound to said receptor, thereby determining whether said compound specifically binds to said receptor.

3. The method according to claim 2, wherein said detecting is performed by monitoring a change in the G- protein coupled signaling activity of said CCR5 chemokine receptor.

4. The method according to claim 3, wherein said detecting is performed by monitoring the level of inositol triphosphate.

5. The method according to claim 3, wherein said detecting is performed by monitoring the level of intracellular calcium in said host cell.

6. The method according to claim 2, wherein said detecting is performed by measuring the modifications of cell metabolism resulting from the stimulation of an intracellular cascade.

8. The method of claim 2, further comprising measuring the infectivity of the cell from said step (c) by HIV in the presence of the detected compound from step (d), wherein a decrease in HIV infectivity of said cell from said step (c) relative to that of said cell from said step (b) which was not exposed to said compound, indicates that said compound inhibits the ability of HIV-1 to utilize said CCR5 chemokine receptor as a cofactor.

9. The method according to claim 8, wherein said infectivity of the cell by HIV is measured by measuring the production of an HIV protein.

12. A method for identifying a compound which specifically binds to the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO: 5, the method comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor. (b) expressing said receptor by said cell. (c) preparing a cell extract from the cell transfected with said nucleic acid molecule, (d) isolating a membrane fraction of said cell extract, (e) contacting said compound with said membrane fraction under conditions permitting binding of the compound to said fraction, and (f) detecting the presence of said compound which has specifically bound to said receptor, wherein said detection indicates that said compound specifically binds to said receptor.

13. A method for identifying a compound as an agonist of the CCR5 chemokine receptor receptor whose amino acid sequence is SEQ ID NO:5, comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor, (b) expressing said receptor under conditions Permitting specific binding of said

compound to said receptor; (c) contacting the cells from part (b) with said compound compound under conditions permitting the activation of a functional peptide response response from the cell, and (d) detecting said response,

wherein the detection of an increase in said response indicates that the compound is an agonist of said CCR5 chemokine receptor.

14. A method for identifying a compound as an antagonist of the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO:5, comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor, (b) expressing said receptor in the transfected cells of part (a), (c) contacting the cells from part (b) with said compound in the presence of an agonist of said receptor, under conditions permitting the activation of a functional response from the cell, and d) detecting said response,

wherein the detection of a decrease in said response relative to the response detected from contacting the cells from part (b) in the presence of said agonist but in the absence of said compound indicates that the compound is an antagonist of said CCR5 chemokine receptor.

☐ 3. Document ID: US 6780598 B1

L25: Entry 3 of 33 File: USPT Aug 24, 2004

DOCUMENT-IDENTIFIER: US 6780598 B1
TITLE: Method of identifying and locating immunobiologically-active linear peptides

CLAIMS:

1. A method for detecting a protein of known sequence comprises the steps of: determining an optimal immunobiologically active linear epitope of said protein of known sequence, wherein said protein is comprised of a plurality of amino acids, and wherein said optimal immunobiologically active linear epitope is characterized by a hydrophobic-hydrophilic-hydrophobic (Ho-Hi-Ho) motif determined by a method comprising the steps of: assigning an average hydropathy value to each of said plurality of amino acids of said protein of known sequence; generating a hydrophilicity plot using said average hydropathy value; fitting each of a plurality of curve segments of said hydrophilicity plot to one of a plurality of a negative cosine functions, wherein one of a plurality of a specific period number values of said plurality of negative cosine functions equates to a particular number of amino acids in one of said plurality of curve segments, said period number value increasing within a predetermined chosen period number range after a sequential lagging of each of said plurality of curve segments through said hydrophilicity plot thereby providing a fit-correlation value for each of said plurality of curve segments across said known sequence of said protein when using one of said plurality of said period number values; generating a potential Ho-Hi-Ho epitope set for each of said plurality of specific period number values within said chosen period number range wherein said potential Ho-Hi-Ho epitope set contains at least one potential Ho-Hi-Ho epitope in which said fit-correlation value is positive; ranking each of said potential Ho-Hi-Ho epitopes of said potential Ho-Hi-Ho epitope set and assigning a ranking value to each of said potential Ho-Hi-Ho

epitopes according to said fit-correlation value wherein said potential Ho-Hi-Ho epitope with a highest positive fit-correlation value is ranked number one, thereby providing a ranked Ho-Hi-Ho potential epitope for each of said plurality of specific specific period number values; examining said ranking value of each of said potential Ho-Hi-Ho epitopes relative to said hydrophilicity plot to determine at least one potential Ho-Hi-Ho epitope set that exhibits alternating positioning around an equilibrium position, wherein a plurality of said ranking values of said potential Ho-Hi-Ho epitopes converge towards or diverge away from said equilibrium position; and designating each of said Ho-Hi-Ho epitopes, wherein said ranking values exhibit a most alternating ranking value that converges or diverges from said said equilibrium position as said optimal immunobiologically active epitope wherein a numeric value of amino acid in said potential Ho-Hi-Ho epitopes is equal to one of of said plutality of specific period number values of said negative cosine function; function; synthesizing at least one peptide corresponding to at least one of said optimal immunobiologically active linear epitopes; creating at least one antisera against said synthesized peptides corresponding to at least one of said optimal immunobiologically active linear epitopes; providing a sample to be analyzed for said protein of known sequence; contacting said sample with said at least one antisera; and detecting a binding of said antisera to said protein of said sample, thereby indicating presence of said protein in said sample.

2. The method of claim 1, wherein said protein is selected from a group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN-.alpha., IFN-.beta.IFN-65, CD2, CD3, CD4, CD5, CD8, CD11A, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54, CD56, K2, K1, P.beta., O.alpha., M.alpha., M.beta.2, M.beta.1, LMP1, TAP2 LMP7, TAP1, O.beta., IA.beta., IA.alpha., IE.beta., IE.beta.2, IE.alpha., CYP21, C4B, CYP21P, C4A, BF, C2, HSP, G7a/b, TNF-.alpha., TNF-.beta., D,L, Qa, Tla, COL11A2, DP.beta.2, DP.alpha.2, DP.beta.1, DP 1, DN 2, DQ 3, DQ 1, DQ 1, DR, DR, HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-E NGF, somtotropin, somatomedins, parathormone, FSH, LH, HEGF, TSH, THS-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF-, GM-CSF, M-CSF, G-CSF1, erythropoietin -ICG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Her2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMEL 17, tyrosine related proteins 1and 2, p97, p53, RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, ras, myc, neu, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl, abil, C1q, C1r, C1s, C4, C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, C1Inh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF, MIRL, CR1, CR2, CR3, CR4, C3a/C4a receptor, HIV (gag, pol, qp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, -TIF, ICP4, ICP8, ICP35, LAT-related proteins, gB, gC, gD, gE, gI, gJ), influenza (hemagluttinin, neuraminidase, PB1, PB2, PA, NP, M.sub.1, M.sub.2, NS.sub.1, NS.sub.2), papillomaviruses (E1, E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2), adenovirus (E1A, E1B, E2, E3, E4, E5, L1, L2, L3, L4, L5), Epstein-Barr Virus (EBNA), Hepatitis B Virus (gp27.sup.s, gp36.sup.s, gp42.sup.s, p22.sup.c, pol, x) and nuclear matrix proteins.

3. A method for detecting a protein of known sequence comprising the steps of: determining an optimal immunobiologically active linear peptide epitope of said protein, wherein said protein is comprised of a plurality of amino acids, wherein said optimal immunobiologically active linear peptide epitope is characterized by fitting a hydrophilicity/hydrophobicity plot generated for said protein of known sequence to a mathematically generated continuous curve thereby generating at least one potential Ho-Hi-Ho epitope which includes at least one ranked potential Ho-Hi-Ho epitope, wherein one of a plurality of a numeric value is assigned to each of said potential Ho-Hi-Ho epitope set corresponding to a fit-correlation value of said hydrophilicity/hydrophobicity plot to said mathematically generated continuous curve, wherein said mathematically generated continuous curve has a period equal to a number of amino acids corresponding to the length of said potential Ho-Hi-Ho

epitope, said mathematically generated curve having a maximum positive value; positioning said at least one ranked potential Ho-Hi-Ho epitope on said hydrophilicity/hydrophobicity plot to determine an oscillating behavior of said plurality of numeric values of said at least one ranked potential Ho-Hi-Ho epitope and deeming each of said plurality of ranked potential Ho-Hi-Ho epitope that exhibit exhibit a most alternating either convergent or divergent positioning about an equilibrium position when juxtaposed on said hydrophilicity/hydrophobicity plot as said optimal immunobiologically active linear peptide epitope, wherein said optimal immunobiologically active linear peptide epitope and its optimal length corresponds to a number of amino acids in the a set of ranked potential Ho-Hi-Ho epitope; synthesizing at least one peptide corresponding to a least one of said optimal immunobiologically active linear epitopes; creating at least one antisera against said synthesized peptides corresponding to at least one of said optimal immunobiologically active linear epitopes; providing a sample to be analyzed for said protein of known sequence; contacting said sample with said at least one antisera; and detecting a binding of said antisera to said protein of said sample, thereby indicating presence of said protein in said sample.

4. The method of claim 3, wherein said protein is selected from a group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN-.alpha., IFN-.beta., IFN65, CD2, CD3, CD4, CD5, CD8, CD11A, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54, CD56, K2, K1, P.beta., O.alpha., M.alpha., M.beta.2, M.beta.1, LMP1, TAP2, LMP7, TAP1, O.beta., IA.beta., IA.alpha., IE.beta., IE.beta.2, E.alpha., CYP21, C4B, CYP21P, C4A, BF, C2, HSP, G7a/b, TNF-.alpha., TNF-.beta., D, L, Qa, TIa, COL11A2, DP.beta.2, DP.alpha.2, DP.beta.1, DN 2, DQ 3, DQ 1, DQ 1, DR , DR , HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-F, NGF, somatotropin, somatomedins, parathormone, FSII, LH, EGF, TSH, THS-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF-, GM-CSF, M-CSF, G-CSFI, erythropoietin, -IICG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Her2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMEL17, tyrosine related proteins 1and 2, p97, p53, RB, APC WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC and MCC ras, myc, neu, raf, erb, src, fms jun, trk ret, gsp, hst, bcl and abil, C1q, C1r, C1s, C4, C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, CUnh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF, MIRL, CR1, CR2, CR3, CR4, C3a/C4a receptor, HIV (gag, pol, qp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, -TIF, ICP4, ICP8, ICP35, LAT-related proteins, gB, gC, gD, gE, gI, gJ), influenza (hemagluttinin, neuraminidase, PB1, PB2, PA, NP, M.sub.1, M.sub.2, NS .sub.1, NS.sub.2), papillomaviruses (E1E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2) adenovirus (E1A, E1B, E2E3, E4, E5, L1, L2, L3, L4, L5), Epstein-Barr Virus (EBNA), Hepatitis B Virus (gp27.sup.s, gp36.sup.s, gp42.sup.s, p22.sup.c, pol, x) nuclear matrix proteins.

☐ 4. Document ID: US 6756035 B2

L25: Entry 4 of 33 File: USPT Jun 29, 2004

DOCUMENT-IDENTIFIER: US 6756035 B2
** See image for Certificate of Correction **
TITLE: Anti-CCR1 antibodies and methods of use therefor

CLAIMS:

14. A method according to claim 12, wherein said humanized antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

15. A method according to claim 14, wherein said humanized antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

17. A method according to claim 16, wherein said recombinant antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

18. A method according to claim 17, wherein said recombinant antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

30. A method according to claim 28, wherein said humanized antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

31. A method according to claim 30, wherein said humanized antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

33. A method according to claim 32, wherein said recombinant antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

34. A method according to claim 33, wherein said recombinant antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

45. A method according to claim 43, wherein said humanized antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

46. A method according to claim 45, wherein said humanized antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

48. A method according to claim 47, wherein said recombinant antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

49. A method according to claim 48, wherein said recombinant antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

51. A method of inhibiting a function associated with binding of a chemokine to a mammalian CC-chemokine receptor 1 (CCR 1) or a functional portion of said receptor, comprising contacting a composition comprising the receptor or functional portion with an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 1 (CCR1) receptor, wherein said antibody or fragment inhibits binding of said chemokine to mammalian CC-chemokine receptor 1 (CCR1) and inhibits one or more functions associated with binding of the chemokine to the receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 2D4 for binding to said receptor.

59. A method according to claim 57, wherein said humanized antibody or antigen-

binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

60. A method according to claim 59, wherein said humanized antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

62. A method according to claim 61, wherein said recombinant antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

63. A method according to claim 62, wherein said recombinant antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

76. A method according to claim 74, wherein said humanized antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

77. A method according to claim 76, wherein said humanized antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

79. A method according to claim 78, wherein said recombinant antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

80. A method according to claim 79, wherein said recombinant antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

92. A method according to claim 90, wherein said humanized antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

93. A method according to claim 92, wherein said humanized antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

95. A method according to claim 94, wherein said recombinant antibody or antigen-binding fragment thereof comprises one or more complementarity-determining regions of monoclonal antibody 2D4.

96. A method according to claim 95, wherein said recombinant antibody or antigen-binding fragment thereof comprises six complementarity-determining regions of monoclonal antibody 2D4.

☐ 5. Document ID: US 6717031 B2

L25: Entry 5 of 33 File: USPT Apr 6, 2004

DOCUMENT-IDENTIFIER: US 6717031 B2

TITLE: Method for selecting a transgenic mouse model of alzheimer's disease

CLAIMS:

1. A method of selecting a transgenic mouse as a model of Alzheimer's disease, comprising providing a plurality of transgenic mice, each comprising a nucleic acid construct stably incorporated into the genome, wherein the construct comprises a promoter for expression of the construct in a mammalian cell and a region encoding an Au-containing protein, wherein the promoter is operatively linked to the region, wherein the region comprises DNA encoding the A.beta.-containing protein, wherein the A.beta.-containing protein consists of all or a contiguous portion of a protein selected from the group consisting of APP770 bearing a mutation in one or more of the amino acids selected from the a group consisting of amino acid 669, 670, 671, 690, 692, and 717, APP751 bearing a mutation in one or more of the amino acids selected from the group consisting of amino acid 669, 670, 671, 690, 692, and 717, and APP695 bearing a mutation in one or more of the amino acids selected from the group consisting of amino acid 669, 670, 671, 690, 692, and 717; a protein consisting of amino acids 672 to 770 of APP; and a protein consisting of amino acids acids 672 to 714 of APP; determining expression levels of APP, APP.beta., APP.alpha. APP.alpha. and/or A.beta. in each of the transgenic mice; identifying a transgenic mouse wherein A.beta.tot is expressed at a level of at least 30 nanograms per gram of brain tissue of the mouse when it is two to four months old, A.beta.1-42 is expressed at a level of at least 8.5 nanograms per gram of brain tissue of the mouse when it is two to four months old, APP and APP.alpha. combined are expressed at a level of a least 150 picomoles per gram of brain tissue of the mouse when it is two to four months old, APP.beta. is expressed at a level of at least 40 picomoles per gram of brain tissue of the mouse when it is two to four months old, and/or mRNA encoding the A.beta.-containing protein is expressed to a level at least twice that of mRNA encoding the cndogenous APP of the transgenic mouse in brain tissue of the mouse when it is two to four months old; using an offspring of the identified transgenic mouse as a model of Alzheimer's disease.

2. The method of claim 1, further comprising administering a compound to be tested to the offspring or cells drived therefrom, and detecting or measuring the Alzheimer's disease marker such that any difference between the marker in the transgenic mouse, or by cells derived from the transgenic mouse rodent, and the marker in a transgenic mouse to which the compound has not been administered, or by cells derived from the transgenic mouse to which the compound has not been administered, is observed, wherein an observed difference in the marker indicates that the compound has an effect on the marker.

4. The method of claim 3 wherein the protein is selected from the group consisting of Cat D,B, Neuronal Thread Protein, nicotine receptors, 5-HT.sub.2 receptor, NMDA receptor, .alpha.2-adrenergic receptor, synaptophysin, p65, glutamine synthetase, glucose transporter, PPI kinase, GAP43, cytochrome oxidase, heme oxygenase, calbindin, adenosine A1 receptors, choline acetyltransferase, acetylcholinesterase, glial fibrillary acidic protein (GFAP), .alpha.1-antitrypsin, C-reactive protein, .alpha.2-macroglobulin, IL-1.alpha., IL-1.beta., TNF.alpha., IL-6, HLA-DR, HLA-A, D,C, CR3 receptor, MHC I, MHC II, CD 31, CR4, CD45, CD64, CD4, spectrin, tau, ubiquitin, MAP-2, apolipoprotein E, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), advanced glycosylation end products, receptor for advanced glycosylation end products, COX-2, CD18, C3, fibroblast growth factor, CD44, ICAM-1, lactotransferrin, C1q, C3d, C4d, C5b-9, gamma RI, Fc gamma RII, CD8, CD59, vitronectin, vitronectin receptor, beta-3 integrin, Apo J, clusterin, type 2 plasminogen activator inhibitor, midkine, macrophage colony stimulating factor receptor, MRP14, 27E10, interferon-alpha, S100.beta., cPLA.sub.2, c-jun, c-fos, HSP27, HSP70, MAP5, membrane lipid peroxidase, protein carbonyl formation, junB, jund, fosB, fra1, cyclin D1, p53, NGFI-A, NGFI-B, I.kappa.B, NF.kappa.B, IL-8, MCP-1, MIP-1.alpha., matrix metaloproteinases, 4-hydroxynonenal-protein conjugates,

amyloid P component, laminin, and collagen type IV.

11. The method of claim 10 wherein the encoded protein is selected from the group consisting of growth inhibitory factor, Cat D,B, Neuronal Thread Protein, nicotine receptors, 5-HT.sub.2 receptor, NMDA receptor, .alpha.2-adrenergic receptor, synaptophysin, p65, glutamine synthetase, glucose transporter, PPI kinase, GAP43, cytochrome oxidase, heme oxygenase, calbindin, adenosine A1 receptors, choline acetyltransferase, acetylcholinesterase, glial fibrillary acidic protein (GFAP), .alpha.1-antitrypsin, C-reactive protein, .alpha.2-macroglobulin, IL-1, TNF.alpha., IL-6, HLA-DR, HLA-A, D,C, CR3 receptor, MHC I, MHC II, CD 31, CR4, CD45, CD64, CD4, spectrin, tau, ubiquitin, MAP-2, apolipoprotein E, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), advanced glycosylation end products, receptor for advanced glycosylation end products, COX-2, CD18, C3, fibroblast growth factor, CD44, ICAM-1, lactotransferrin, C1q, C3d, C4d, C5b-9, gamma RI, Fc gamma RII, CD8, CD59, vitronectin, vitronectin receptor, beta-3 integrin, Apo J, clusterin, type 2 plasminogen activator inhibitor, midkine, macrophage colony stimulating factor receptor, MRP14, 27E10, interferon-alpha, S100.beta., cPLA.sub.2, c-jun, c-fos, HSP27, HSP70, MAP5, membrane lipid peroxidase, protein carbonyl formation, junB, junD, fosB, fra1, cyclin D1, p53, NGFI-A, NGFI-B, I.kappa.B, NF.kappa.B, IL-8, MCP-1, MIP-1.alpha., matrix metaloproteinases, 4-hydroxynonenal-protein conjugates, amyloid P component, laminin, and collagen type IV.

33. A method of selecting a transgenic mouse ha a model of Aizheimer's disease, comprising providing a plurality of transgenic mice, each comprising a nucleic acid construct stably incorporated into the genome, wherein the construct comprises a promoter for expression of the construct in a mammalian cell and a region encoding an A.beta.-containing protein, wherein the promoter is operatively linked to the region, wherein the region comprises DNA encoding the A.beta.-containing protein, wherein the A.beta.-containing protein consists of all or a contiguous portion of a protein selected from the group consisting of APP770 bearing a mutation in one or more of the amino acids selected from the group consisting of amino acid 669, 670, 671, 690, 692, and 717, APP751 bearing a mutation in one or more of the amino acids selected from the group consisting of amino acid 669, 670, 671, 690, 692, and 717, and APP695 bearing a mutation in one or more of the amino acids selected from the group consisting of amino acid 669, 670, 671, 690, 692, and 711, a protein consisting of amino acids 672 to 770 of APP; and a protein consisting of amino acids 672 to 714 of APP; determining expression levels of A.beta. and Congo red staining in the brains of each of the transgenic mice; identifying a transgenic mouse; wherein A.beta. is expressed at a level of at least 50 ng/g brain tissue in the identified transgenic mouse when the transgenic mouse is three months of age; and using an offspring of the identical transgenic mouse as a model of Alzheimer's Disease.

34. The method of claim 33, further comprising administering a compound to be tested to the offspring or cells derived therefrom, and detecting or measuring the Alzheimer's disease marker such that any difference between the marker in the transgenic mouse, or by cells derived from the transgenic mouse rodent, and the marker in a transgenic mouse to which the compound has not been administered, or by cells derived from the transgenic mouse to which the compound has not been administered, is observed, wherein an observed difference in the marker indicates that the compound has an effect on the marker.

42. The method of claim 41 wherein the protein is selected from the group consisting of growth inhibitory factor, Cat D,B, Neuronal Thread Protein (CSF), nicotine receptors, 5-HT.sub.2 receptor, NMDA receptor, .alpha.2-adrenergic receptor, synaptophysin, p65, glutamine synthetase, glucose transporter, PPI kinase, GAP43, cytochrome oxidase, calbindin, adenosine A1 receptors, choline acetyltransferase, acetylcholinesterase, glial fibrillary acidic protein (GFAP), .alpha.1-antichymotrypsin, .alpha.1-antitrypsin, C-reactive protein, .alpha.2-

macroglobulin, IL-1, TNF.alpha., IL-6, HLA-DR, HLA-A, D,C, CR3 receptor, MHC I, MHC II, CD 31, CR4, CD45, CD64, CD4, spectrin, tall, ubiquitin, MAP-2, apolipoprotein E, E, glycosylation end products, amyloid P component, laminen, and collagen type IV.

☐ 6. Document ID: US 6646109 B1

L25: Entry 6 of 33 File: USPT Nov 11, 2003

DOCUMENT-IDENTIFIER: US 6646109 B1
TITLE: Cloning and expression of a novel acetylcholine-gated ion channel receptor subunit

CLAIMS:

9. A method for detecting the presence of alpha9 nicotinic acetylcholine receptor on on the surface of a cell, said method comprising: contacting the cell with an antibody according to claim 7 and assaying for the presence of said antibody bound to the cell surface.

10. A method for identifying the location of an alpha9 nicotinic acetylcholine receptor on the surface of a cell, said method comprising: contacting the cell with an antibody according to claim 7, identifying the presence and location of said antibody on the cell surface.

11. A method for detecting the presence of alpha9 nicotinic acetylcholine receptor on the surface of a cell, said method comprising: contacting the cell with an antibody according to claim 1 and assaying for the presence of said antibody bound to the cell surface.

12. A method for identifying the location of an alpha9 nicotinic acetylcholine receptor on the surface of a cell, said method comprising: contacting the cell with an antibody according to claim 1, and identifying the presence and location of said antibody on the cell surface.

13. A method for modulating the ion channel activity of an alpha9 nicotinic acetylcholine receptor, said method comprising: contacting a cell, having on its surface alpha9 nicotinic acetylcholine receptor, with a composition comprising a carrier and an amount of an antibody according to claim 1 effective to block the binding of naturally occurring ligands to said receptor.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 7. Document ID: US 6569865 B2

L25: Entry 7 of 33 File: USPT May 27, 2003

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☐ 1. Document ID: US 6680170 B2

L10: Entry 1 of 12 File: USPT Jan 20, 2004

DOCUMENT-IDENTIFIER: US 6680170 B2
TITLE: Polynucleotides encoding STE20-related protein kinases and methods of use

Brief Summary Text (31):
The term "signal transduction pathway" refers to the molecules that propagate an extracellular signal through the cell membrane to become an intracellular signal. This signal can then stimulate a cellular response. The polypeptide molecules involved in signal transduction processes are typically receptor and non-receptor protein tyrosine kinases, receptor and non-receptor protein phosphatases, SRC homology 2 and 3 domains, phosphotyrosine binding proteins (SRC homology 2 (SH2) and phosphotyrosine binding (PTB and PH) domain containing proteins), proline-rich binding proteins (SH3 domain containing proteins), nucleotide exchange factors, and transcription factors.

Brief Summary Text (103):
"Amplification" as it refers to RNA can be the detectable presence of kinase RNA in cells, since in some normal cells there is no basal expression of kinase RNA. In other normal cells, a basal level of expression of kinase exists, therefore in these these cases amplification is the detection of at least 1-2-fold, and preferably more, kinase RNA, compared to the basal level.

Detailed Description Text (262):
The present invention also encompasses a method of detecting a STE20-related kinase polypeptide in a sample, comprising: (a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and (b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of a kinase of the invention in a sample as compared to normal levels levels may indicate disease.

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw De

☐ 2. Document ID: US 6656716 B1

L10: Entry 2 of 12 File: USPT Dec 2, 2003

DOCUMENT-IDENTIFIER: US 6656716 B1

TITLE: Polypeptide fragments of human PAK5 protein kinase

Brief Summary Text (32):
The term "signal transduction pathway" refers to the molecules that propagate an extracellular signal through the cell membrane to become an intracellular signal. This signal can then stimulate a cellular response. The polypeptide molecules involved in signal transduction processes are typically receptor and non-receptor protein tyrosine kinases, receptor and non-receptor protein phosphatases, SRC homology 2 and 3 domains, phosphotyrosine binding proteins (SRC homology 2 (SH2) and phosphotyrosine binding (PTB and PH) domain containing proteins), proline-rich binding proteins (SH3 domain containing proteins), nucleotide exchange factors, and transcription factors.

Brief Summary Text (104):
"Amplification" as it refers to RNA can be the detectable presence of kinase RNA in cells, since in some normal cells there is no basal expression of kinase RNA. In other normal cells, a basal level of expression of kinase exists, therefore in these these cases amplification is the detection of at least 1-2-fold, and preferably more, kinase RNA, compared to the basal level.

Detailed Description Text (264):
The present invention also encompasses a method of detecting a STE20-related kinase polypeptide in a sample, comprising: (a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and (b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of a kinase of the invention in a sample as compared to normal levels levels may indicate disease.

☐ 3. Document ID: US 6495376 B1

L10: Entry 3 of 12 File: USPT Dec 17, 2002

DOCUMENT-IDENTIFIER: US 6495376 B1
**** See image for Certificate of Correction ****
TITLE: Methods and compositions for regulating protein-protein interactions

Detailed Description Text (67):
SH2 domains have been demonstrated to be critical for generating processive phosphorylation by nonreceptor tyrosine kinases (Songyang, Z. et al. Nature 373:536 (1995); Mayer, B. J. et al., Curr. Biol. 5:296 (1995)). SH2 domains in these kinases prefer to bind phosphotyrosine residues that have been phosphorylated by its own catalytic domain. The resulting high phosphorylation of substrates on multiple sites (Songyang, Z. et al. Nature 373:536(1995); Mayer, B. J. et al., Curr. Biol. 5:296 (1995)). WW-domains can facilitate the processive isomerization of proteins that have been phosphorylated by mitotic kinases at multiple sites. The processive isomerization is triggered by binding of the higher affinity WW-domain of Pin1 to a Ser-phosphorylated site on a substrate protein. Once bound, the high local concentration drives isomerization of all sites that are sterically accessible to the lower affinity catalytic PPIase domain. This can provide a means by which to generate coordinate "all-or-nothing" activity of mitotic

phosphoproteins and subsequently sequential mitotic events.

Detailed Description Text (142):
Binding of Pin1 to PHFs could trap Pin1 in the tangles, eventually leading to depletion of the soluble Pin1 in neurons. To test this possibility, the levels of Pin1 and two tau kinases, GSK3b and Cdc2, were compared in AD and normal brain tissues. Brain tissues were homogenized and soluble proteins were directly subjected to immunoblotting analysis, followed by semi-quantification of protein levels using ImageQuan. When compared with 6 age-matched normal brains, GSK3b levels were slightly reduced (40.+-.11%), and Cdc2 levels were significantly increased by approximately 5 fold in AD brains (547.+-.87%, n=6, P<0.01). These findings are consistent with previous studies showing that levels of Cdc2, but not GSK3b, are abnormally elevated in Alzheimer's disease brains (Vincent, I. et al., J Neurosci 17:3588-3598 (1997)).

☐ 4. Document ID: US 6368796 B1

L10: Entry 4 of 12 File: USPT Apr 9, 2002

DOCUMENT-IDENTIFIER: US 6368796 B1
** See image for Certificate of Correction **
TITLE: Methods of detection and treatment of breast cancer

Detailed Description Text (2):
The family of protein tyrosine kinases (PTKs) includes oncogenes and growth factor receptors, several of which have been linked to the pathogenesis and progression of certain cancers (Bishop, J. M., Genes. Dev., 9:1309-1315 (1995)Cancer, W. G., et al., Breast Can. Res. & Treat., 35:105-1114 (1995)). Increasing evidence indicates that the c-src proto-oncogene may play an important role in breast cancer. Human breast cancers often show much higher levels of src protein kinase activity than normal adjacent epithelium (Hennipman, A., et al., Cancer Research, 49:516-521 (1989), Ottenhoff-Kalff, A. E., et al., Cancer Research, 52:4773-4778 (1992)). Indeed, about 70% of the elevated total tyrosine kinase activity found in primary breast cancers can be attributed to increased src activity. Involvement of pp60c-src with two major signaling pathways in human breast cancer has been demonstrated. In human breast carcinoma cell lines, the SH2 domain of src binds to activated epidermal growth factor (EGF-R) and p.sub.185.sup.ErbB- 2, a receptor tyrosine kinase (Luttrell, D. K., et al., Proc. Natl. Acad. Sci. USA, 91:83-87 (1994)).

Other Reference Publication (13):
Hamaguchi, I. et al., "Characterization of mouse non-receptor tyrosine kinase gene, HYL," Oncogene 9:3371-3374 (1994).

Other Reference Publication (17):
Kuo, S.S. et al., "Identification and Characterization of Batk, a Predominantly Brain-Specific Non-Receptor Protein Tyrosine Kinase Related to Csk," J. Neurosci. Res. 38:705-715 (1994).

Other Reference Publication (27):
Sakano, S. et al., "Molecular cloning of a novel non-receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase)," Oncogene 9:1155-1161 (1994).

☐ 5. Document ID: US 6210654 B1

L10: Entry 5 of 12 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210654 B1
TITLE: Jak kinases and regulation of cytokine signal transduction

Brief Summary Text (13):
Tyrosine phosphorylation has further been associated with the response to the cytokine growth hormone (GH). Studies in 3T3-F442A cells showing rapid GH-dependent tyrosyl phosphorylation of multiple proteins, tyrosyl phosphorylation of microtubule-associated protein kinases, and stimulation of microtubule-associated protein kinase activity, as well as the inhibition of these actions by inhibitors of growth hormone receptor (GHR)-associated tyrosine kinase (Campbell et al., J. Biol. Chem. 268:7427-7434 (1993)), suggest a central role for a GHR-associated tyrosine kinase activity in signaling by GH. In addition, the presence of a tyrosine kinase activity in a complex with GH receptor (GHR) prepared from GH-treated fibroblasts has been reported (Carter-Su. et al., J. Biol. Chem. 264:18654-18661 (1989); Stred et al., Endocrinol. 130: 1626-1636 (1992); Wang et al., J. Biol. Chem. 267: 17390-17396 (1992)). More recently, a nonreceptor tyrosyl phosphorylated 122 kd protein was identified in a kinase-active GH-GHR preparation (Wang et al., J. Biol. Chem. 268:3573-3579 (1993)).

Brief Summary Text (16):
Two of the other tyrosine kinases expressed in IL-3-dependent cells, Jak1 and Jak2, belong to the Jak family of kinases. The Jak (Janus kinase; alternatively referred to as just another kinase) family of kinases were initially detected in PCR amplification of tyrosine kinase domains in hematopoietic cells (Wilks, Proc. Natl. Acad. Sci. USA 86:1603-1607 (1989)). These studies identified two closely related genes (FD17 and FD22; later termed Jak2 and Jak1) from which the major PCR amplification products were derived. The complete structure of the human Jak1 gene has been reported (Wilks et al., Mol. Cell. Biol. 11:2057-2065 (1991)) and, recently, a partial sequence of the murine Jak2 gene was published (Harpur et al., Oncogene 7:1347-1353 (1992)). Independently a third member of the family (Tyk2) was isolated by screening a cDNA library with a tyrosine kinase domain probe from the c-fms gene (Firmbach-Kraft et al., Oncogene 5:1329-1336 (1990)). The family is characterized by the presence of two kinase domains, one of which is a carboxyl domain that has all the hallmarks of protein kinases. The second domain is immediately amino terminal and bears all the hallmarks of a protein kinase but differs significantly from both the protein tyrosine and serine/threonine kinases. Amino terminal to the kinase domains, there are no SH2 and SH3 domains that characterize most of the non-receptor tyrosine kinases. However, there is extensive similarity in this region among the Jak family members and a number of homology domains have been defined (Harpur et al., Oncogene 7:1347-1353 (1992)).

Brief Summary Text (28):
A possible clue to the identity of the cytoplasmic tyrosine kinase(s) activated by the CNTF family of factors came from the finding that other distantly related cytokines resulted in the activation of the Jak/Tyk family of kinases (Firmbach-Kraft et al., Oncogene 5:1329-1336 (1990); Wilks et al., Mol. Cell. Biol. 11:2057-2065 (1991); Harpur et al., Oncogene 7:1347-1353 (1992)). This family of nonreceptor cytoplasmic protein tyrosine kinases consists of 3 known members--Jak1, Jak2, and Tyk2--which are all equally related to each other and share the unusual feature of having two potential kinase domains and no Src homology 2 (SH2) domains.

Elegant studies involving complementation of a genetic defect in a cell line unresponsive to IFNa resulted in the identification of Tyk2 as a required component of the IFN.alpha. signaling cascade ((Velasquez et al., Cell 70:313-322 (1992)). More recently, the receptors for cytokines such as EPO, GM-CSF, and GH were shown to to associate with and activate Jak2 (Argetsinger et al., Cell 74:237-244 (1993); Silvennoinen et al., Proc. Natl. Acad. Sci. USA (1993, in press); Witthuhn et al., Cell 74:227-236 (1993)). The kinase was shown to bind to the membrane proximal cytoplasmic region of the receptor, and mutations of this region that prevented Jak2 Jak2 binding also resulted in the loss of EPO induced proliferation, suggesting that that Jak2 plays a critical role in EPO signaling. Jak1 has not been reported to be significantly activated by any of these receptor systems.

Detailed Description Text (56):
In those situations where the biological response of a cell to a cytokine is deficient due to insufficient amounts of a Jak kinase, the present invention provides for enhancing this response by increasing the levels of the Jak kinase in the cell (see Example 4). This situation could be due to mutations which reduce the amount of the Jak kinase produced by the cell to sub-normal levels. This situation could also be due to mutations which reduce the rate or degree of cytokine-induced Jak activation such that the level of Jak kinase produced by the cell does not provide sufficient levels of activated Jak kinase following cytokine induction.

Detailed Description Text (108):
The Jak (Janus kinase; alternatively referred to as just another kinase) family of kinases was initially detected in PCR amplification of tyrosine kinase domains in hematopoietic cells (Wilks, A. F., Proc. Natl. Acad. Sci. USA 86:1603-1607 (1989)). These studies identified two closely related genes (FD17 and FD22; later termed Jak2 and Jak1) from which the major PCR amplification products were derived. The complete structure of the human Jak1 gene has been reported (Wilks, A. F., et al., Mol. Cell. Biol. 11:2057-2065 (1991)) and, recently, a partial sequence of the murine Jak2 gene was published (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)). Independently a third member of the family (Tyk2) was isolated by screening a cDNA library with a tyrosine kinase domain probe from the c-fms gene (Firmbach-Kraft, I., et al., Oncogene 5:1329-1336 (1990)). The family is characterized by the presence of two kinase domains, one of which is a carboxyl domain that has all the hallmarks of protein kinases: The second domain is immediately amino terminal and bears all the hallmarks of a protein kinase but differs significantly from both the protein tyrosine and serine/threonine kinases. Amino terminal to the kinase domains, there are no SH2 and SH3 domains that characterize most of the non-receptor tyrosine kinases. However, there is extensive similarity in this region among the Jak family members and a number of homology domains have been defined (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)).

Detailed Description Text (224):
Growth hormone receptor (GHR) forms a complex with a tyrosine kinase, suggesting involvement of a ligand-activated tyrosine kinase in intracellular signaling by growth hormone (GH). Here we identify Jak2, a nonreceptor tyrosine kinase, as a GHR-associated tyrosine kinase. Immunological approaches were used to establish GH-dependent complex formation between Jak2 and GHR, activation of Jak2 tyrosine kinase activity, and tyrosyl phosphorylation of both Jak2 and GHR. The Jak2-GHR and Jak2-erythropoietin receptor interactions described here and in the accompanying Example 2 provide a molecular basis for the role of tyrosyl phosphorylation in physiological responses to these ligands, thus evidencing shared signaling mechanism among members of the cytokine/hematopoietin receptor family.

Detailed Description Text (226):
Although the ability of growth hormone (GM) to promote growth and regulate metabolism has been known for many years (Cheek, D. B. and Hill, D. E., "Effect of growth hormone on cell and somatic growth," in E. Knobli and W. H. Sawyer, eds., Handbook of Physiology, Vol. 4:159-185, Washington, D.C. (1974); Davidson, M. B.,

Rev. 8:115-131 (1987)), the molecular mechanism by which GH binding to its receptor elicits its diverse responses has remained an enigma. New insight into GH signaling mechanisms was recently provided by the demonstration that a tyrosine kinase activity is present in a complex with GH receptor (GHR) prepared from GH-treated fibroblasts (Carter-Su., C. et al., J. Biol. Chem. 264:18654-18661 (1989); Stred, S. S. E. et al., Endocrinol. 130:1626-1636 (1992); Wang, X. et al., J. Biol. Chem. 267: 267: 17390-17396 (1992)). Additional studies in 3T3-F442A cells showing rapid GH-dependent tyrosyl phosphorylation of multiple proteins, tyrosyl phosphorylation of microtubule-associated protein kinases, and stimulation of microtubule-associated protein kinase activity, as well as the inhibition of these actions by inhibitors of of the GHR-associated tyrosine kinase (Campbell, G. S. et al., J. Biol. Chem. 268:7427-7434 (1993)), suggest a central role for a GHR-associated tyrosine kinase in signaling by GH. Recently, a nonreceptor tyrosyl phosphorylated 122 kd protein was identified in a kinase-active GH-GHR preparation (Wang, X. et al., J. Biol. Chem. 268:3573-3579 (1993)). Since autophosphorylation is often a manifestation of an activated kinase, it was hypothesized that this 121 kd phosphoprotein is the GHR-associated kinase.

Other Reference Publication (10):
Firmbach-Kraft, I. et al., "tyk2, prototype of a novel class of non-receptor tyrosine kinase genes," Oncogene 5:1329-1336 (1990).

☐ 6. Document ID: US 6136581 A

L10: Entry 6 of 12 File: USPT Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136581 A
TITLE: Kinase genes and uses

Brief Summary Text (5):
Cellular signal transduction is a fundamental mechanism whereby external stimuli that regulate diverse cellular processes are relayed to the interior of cells. One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of proteins, which enables regulation of the activity of mature proteins by altering their structure and function. For reviews, see Posada and Cooper, Mol. Biol. Cell, 3:583-392 (1992) and Hardie, Symp. Soc. Exp. Biol. 44:241-255 (1990)). The best characterized protein kinases in eukaryotes phosphorylate proteins on the alcohol moiety of serine, threonine and tyrosine residues. These kinases largely fall into two groups, those specific for phosphorylating serines and threonines, and those specific for phosphorylating tyrosines. The tyrosine kinases can be further divided into receptor and non-receptor proteins.

Brief Summary Text (8):
The non-receptor tyrosine kinases do not contain a transmembrane domain or an extracellular domain and share non-catalytic domains in addition to sharing their catalytic kinase domains. Such non-catalytic domains include the SH2 domain (Src homology domain 2) and SH3 domains (Src homology domain 3). The non-catalytic domains are thought to be important in the regulation of protein-protein interactions during signal transduction.

Detailed Description Text (25):
Many human genes are expressed at different levels in different tissues. In some cases, a gene-is not expressed at all in some cells or tissues, and at high levels

in others. Thus, a variety of different human cell lines from various tissue sources, as well as several different normal human tissues were analyzed for the expression levels of the tyrosine kinase genes identified in this invention. In general, the expression level of a gene was determined by determination of the amount of the corresponding messenger RNA (mRNA) present in the cells, based on hybridization with a labeled probed under specific hybridization conditions.

Detailed Description Text (74):
The present invention encompasses a method of detecting a polypeptide encoded by one of the disclosed genes in a sample, by: (a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and (b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods involves incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of a kinase in a sample as compared to normal levels may indicate disease.

☐ 7. Document ID: US 6100386 A

L10: Entry 7 of 12 File: USPT Aug 8, 2000

DOCUMENT-IDENTIFIER: US 6100386 A
**** See image for Certificate of Correction ****
TITLE: Human gene/protein involved in chronic myelogenous leukemia

Brief Summary Text (3):
Compared to normal c-abl, bcr-abl has increased tyrosine kinase activity. Konopka, J., et al., Cell, 37:1035-1042 (1984). Additionally, c-abl, as a non-receptor tyrosine kinase, functions both in the nucleus and the cytoplasm and bcr-abl functions exclusively in the cytoplasm. These two characteristics of bcr-abl are essential elements of its transforming abilities. McWhirter, J. R., et al., Mol. Cell Bio., 11:1553-1565 (1991).

Brief Summary Text (13):
The present invention also relates to host cells which contain p62.sup.dok - encoding nucleic acid (DNA, RNA) and express the p62.sup.dok protein. The host cell may, optionally, express an oncogenic tyrosine kinase at levels which are elevated, the same as or decreased, relative to activity of a normal (non-oncogenic) tyrosine kinase. In a particular embodiment, the host cell comprises nucleic acid encoding the p62.sup.dok protein of the present invention operably linked to an expression control sequence; the encoded p62.sup.dok protein is expressed when the host cell is maintained under conditions suitable for expression.

Other Reference Publication (15):
Neet, K. and Hunter, T., "The nonreceptor protein-tyrosine kinase csk complexes directly with the GTPase-activating protein-associated p62 in cells expressing v-src or activated c-src," Mol. Cell Biol., 15:4908-4920 (1995).

☐ 8. Document ID: US 6090621 A

L10: Entry 8 of 12 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090621 A
TITLE: Signaling inositol polyphosphate 5-phosphatases (SIPs)

Brief Summary Text (5):
SHC and GRB2 protein signaling molecules form a complex in response to growth factor or oncogenic transformation, as described in Rozakis-Adcock et al Nature 360: 689-92 (1992). These proteins are thought to transmit mitogenic signals from receptor and non-receptor tyrosine kinases to ras, a member of a major class of oncogenes and proto-oncogenes that encode G proteins that are located on the inner face of the plasma membrane, where they bind and hydrolyze GTP. Ras proteins are involved in an unknown way in growth-factor stimulation of cell proliferation, as described in Alberts et al MOLECULAR BIOLOGY OF THE CELL (second edition, Garland publishing New York, 1989) pp. 699 and 705. The precise mechanism of the action of ras remains unknown, as indicated in Lowenstein et al Cell 70: 431-42 (1992) and Gale et al Nature 363: 88-92 (1993).

Brief Summary Text (12):
The invention is also a method of treating a mammal having or at risk for having a condition characterized by higher than normal levels of PI 3-kinase activity by providing a therapeutic agent including a SIP polypeptide, a polynucleotide encoding a SIP polypeptide, or a modulator of a SIP polypeptide, and administering an effective amount of this therapeutic agent to a mammal.

Brief Summary Text (13):
The invention is also method of treating a mammal having or at risk for having a condition characterized by higher than normal levels of mitogen activated protein (MAP) kinase activity by providing a therapeutic agent including a SIP polypeptide, a polynucleotide encoding a SIP polypeptide, or a modulator of a SIP polypeptide, and administering an effective amount of this therapeutic agent to a mammal.

Brief Summary Text (17):
The invention is a pharmaceutical composition for treating a mammal having a population of mitogenic cells having higher than normal levels of PI 3-kinase activity or higher than normal levels of MAP kinase activity using an effective amount of a therapeutic agent including a SIP polypeptide, a polynucleotide encoding a SIP polypeptide, a modulator of a SIP polypeptide, and a pharmaceutically acceptable carrier.

Brief Summary Text (22):
It is also an object of the invention to provide a method of treating a mammal having or at risk for having a condition characterized by lower than normal levels of PI 3-kinase activity by providing a therapeutic agent that is an antagonist of a SIP polypeptide, and administering an effective amount of this therapeutic agent to the mammal. A further object of the invention is a method of treating a mammal having or at risk for having a condition characterized by lower than normal levels of mitogen activated protein (MAP) kinase activity by providing a therapeutic agent that is an antagonist of a SIP polypeptide, and administering an effective amount of this therapeutic agent to the mammal. The invention is also a method of increasing a level of phosphatidylinositol (3,4,5) triphosphate (PtdIns(3,4,5) P.sub.3) in a cell in a mammal by providing a therapeutic agent that is an antagonist of a SIP polypeptide, and administering an effective amount of this therapeutic agent to the mammal. Another object is a method of stimulating cell growth in a population of cells in a mammal by providing a therapeutic agent that

is an antagonist of a SIP polypeptide, and administering an effective amount of this this therapeutic agent to the mammal.

Brief Summary Text (23):
Another object is a pharmaceutical composition for treating a mammal having a population of cells having lower than normal levels of PI 3-kinase activity or lower lower than normal levels of MAP kinase activity having an effective amount of a therapeutic agent that is an antagonist of a SIP polypeptide and a pharmaceutically acceptable carrier.

Detailed Description Text (66):
Thus, the inventors have discovered a method reducing a level of PI 3kinase activity in a cell having higher than normal levels of PI 3-kinase activity by providing a therapeutic agent including either a SIP polypeptide or a modulator of a SIP polypeptide, or a polynucleotide encoding a SIP polypeptide, and contacting the cell with an effective amount of the therapeutic agent.

Detailed Description Text (67):
The invention also is a method reducing a level of mitogen activated protein (MAP) kinase activity in a cell having higher than normal levels of MAP kinase activity by providing a therapeutic agent including a SIP polypeptide, a polynucleotide encoding a SIP polypeptide, and a modulator of a SIP polypeptide, and contacting the cell with an effective amount of the therapeutic agent.

Detailed Description Text (70):
The invention is also a pharmaceutical composition for treating a mammal having a population of mitogenic cells having higher than normal levels of PI 3-kinase activity or higher than normal levels of MAP kinase activity including an effective amount of a therapeutic agent including a SIP polypeptide, a polynucleotide encoding a SIP polypeptide, a polypeptide encoding a functional portion of the sequence of SEQ ID No. 15, or a modulator of a functional portion of the sequence of SEQ ID No. 15, and administering an effective amount of this therapeutic agent to the mammal. This pharmaceutical composition can include a therapeutic agent that is a modulator of a functional portion of the sequence of SEQ ID No. 15, and this modulator can be a polynucleotide, a polypeptide, a small molecule, a peptide, or a peptoid. The pharmaceutical composition can include a modulator that is a polynucleotide and the polynucleotide can be a coding sequence or a noncoding sequence. The pharmaceutical composition can be a polynucleotide modulator of SIP that is a noncoding sequence, and the noncoding sequence can be a 3' untranslated region, a 5' untranslated region, an antisense oligonucleotide, or a ribozyme.

Detailed Description Text (74):
The invention is a method of increasing a level of PI 3-kinase activity in a cell having lower than normal levels of PI 3-kinase activity by providing a therapeutic agent that is an antagonist of a SIP polypeptide, and contacting the cell with an effective amount of the therapeutic agent.

Detailed Description Text (75):
Likewise, the invention is also a method increasing a level of MAP kinase activity in a cell having lower than normal levels of MAP kinase activity by providing a therapeutic agent that is an antagonist of a SIP polypeptide, and contacting the cell with an effective amount of the therapeutic agent.

Detailed Description Text (78):
The invention is also a pharmaceutical composition for treating a mammal having a population of cells having lower than normal levels of PI 3-kinase activity or lower lower than normal levels of MAP kinase activity having an effective amount of a therapeutic agent that is an antagonist of a SIP polypeptide and a pharmaceutically acceptable carrier. The pharmaceutical composition just described can include an antagonist of a SIP polypeptide that is a polynucleotide, a polypeptide, a small

molecule, a peptide, or a peptoid. Further, the antagonist can be a polynucleotide that is a coding sequence or a noncoding sequence. The polynucleotide that is a noncoding sequence, can be a 3' untranslated region, a 5' untranslated region, an intron, an antisense oligonucleotide, or a ribozyme.

☐ 9. Document ID: US 5981201 A

L10: Entry 9 of 12 File: USPT Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981201 A
** See image for Certificate of Correction **
TITLE: Methods of detection and treatment of breast cancer

Detailed Description Text (2):
The family of protein tyrosine kinases (PTKs) includes oncogenes and growth factor receptors, several of which have been linked to the pathogenesis and progression of certain cancers (Bishop, J. M., Genes. Dev., 9:1309-1315 (1995)Cancer, W. G., et al., Breast Can. Res. & Treat., 35:105-1114 (1995)). Increasing evidence indicates that the c-src proto-oncogene may play an important role in breast cancer. Human breast cancers often show much higher levels of src protein kinase activity than normal adjacent epithelium (Hennipman, A., et al., Cancer Research, 49:516-521 (1989), Ottenhoff-Kalff, A. E., et al., Cancer Research, 52:4773-4778 (1992)). Indeed, about 70% of the elevated total tyrosine kinase activity found in primary breast cancers can be attributed to increased src activity. Involvement of pp60c-src with two major signaling pathways in human breast cancer has been demonstrated. In human breast carcinoma cell lines, the SH2 domain of src binds to activated epidermal growth factor (EGF-R) and p.sub.185.sup.ErbB-2, a receptor tyrosine kinase (Luttrell, D. K., et al., Proc. Natl. Acad. Sci. USA, 91:83-87 (1994)).

Other Reference Publication (12):
Hamaguchi, I. et al., "Characterization of mouse non-receptor tyrosine kinase gene, HYL," Oncogene 9:3371-3374 (1994).

Other Reference Publication (16):
Kuo, S.S. et al., "Identification and Characterization of Batk, a Predominantly Brain-Specific Non-Receptor Protein Tyrosine Kinase Related to Csk," J. Neurosci. Res. 38:705-715 (1994).

Other Reference Publication (26):
Sakano, S. et al., "Molecular cloning of a novel non-receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase)," Oncogene 9:1155-1161 (1994).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 10. Document ID: US 5728536 A

L10: Entry 10 of 12 File: USPT Mar 17, 1998

DOCUMENT-IDENTIFIER: US 5728536 A
** See image for Certificate of Correction **
TITLE: Jak kinases and regulation of Cytokine signal transduction

Brief Summary Text (13):
Tyrosine phosphorylation has further been associated with the response to the cytokine growth hormone (GH). Studies in 3T3-F442A cells showing rapid GH-dependent tyrosyl phosphorylation of multiple proteins, tyrosyl phosphorylation of microtubule-associated protein kinases, and stimulation of microtubule-associated protein kinase activity, as well as the inhibition of these actions by inhibitors of growth hormone receptor (GHR)-associated tyrosine kinase (Campbell, G. S. et al., J. Biol. Chem. 268:7427-7434 (1993)), suggest a central role for a GHR-associated tyrosine kinase activity in signaling by GH. In addition, the presence of a tyrosine kinase activity in a complex with GH receptor (GHR) prepared from GH-treated fibroblasts has been reported (Carter-Su., C. et al., J. Biol. Chem. 264:18654-18661 (1989); Stred, S. E. et al., Endocrinol. 130:1626-1636 (1992); Wang, X. et al., J. Biol. Chem. 267:17390-17396 (1992)). More recently, a nonreceptor tyrosyl phosphorylated 122 kd protein was identified in a kinase-active GH-GHR preparation (Wang, X. et al., J. Biol. Chem. 268:3573-3579 (1993)).

Brief Summary Text (16):
Two of the other tyrosine kinases expressed in IL-3-dependent cells, Jak1 and Jak2, belong to the Jak family of kinases. The Jak (Janus kinase; alternatively referred to as just another kinase) family of kinases were initially detected in PCR amplification of tyrosine kinase domains in hematopoietic cells (Wilks, A. F., Proc. Natl. Acad. Sci. USA 86:1603-1607 (1989)). These studies identified two closely related genes (FD17 and FD22; later termed Jak2 and Jak1) from which the major PCR amplification products were derived. The complete structure of the human Jak1 gene has been reported (Wilks, A. F., et al., Mol. Cell. Biol. 11:2057-2065 (1991)) and, recently, a partial sequence of the murine Jak2 gene was published (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)). Independently a third member of the family (Tyk2) was isolated by screening a cDNA library with a tyrosine kinase domain probe from the c-fms gene (Firmbach-Kraft, I., et al., Oncogene 5:1329-1336 (1990)). The family is characterized by the presence of two kinase domains, one of which is a carboxyl domain that has all the hallmarks of protein kinases. The second domain is immediately amino terminal and bears all the hallmarks of a protein kinase but differs significantly from both the protein tyrosine and serine/threonine kinases. Amino terminal to the kinase domains, there are no SH2 and SH3 domains that characterize most of the non-receptor tyrosine kinases. However, there is extensive similarity in this region among the Jak family members and a number of homology domains have been defined (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)).

Detailed Description Text (31):
In those situations where the biological response of a cell to a cytokine is deficient due to insufficient amounts of a Jak kinase, the present invention provides for enhancing this response by increasing the levels of the Jak kinase in the cell (see Example 4). This situation could be due to mutations which reduce the amount of the Jak kinase produced by the cell to sub-normal levels. This situation could also be due to mutations which reduce the rate or degree of cytokine-induced Jak activation such that the level of Jak kinase produced by the cell does not provide sufficient levels of activated Jak kinase following cytokine induction.

Detailed Description Text (72):
The Jak (Janus kinase; alternatively referred to as just another kinase) family of kinases was initially detected in PCR amplification of tyrosine kinase domains in hematopoietic cells (Wilks, A. F., Proc. Natl. Acad. Sci. USA 86:1603-1607 (1989)). These studies identified two closely related genes (FD17 and FD22; later termed Jak2 and Jak1) from which the major PCR amplification products were derived. The

complete structure of the human Jak1 gene has been reported (Wilks, A. F., et al., Mol. Cell. Biol. 11:2057-2065 (1991)) and, recently, a partial sequence of the murine Jak2 gene was published (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)). (1992)). Independently a third member of the family (Tyk2) was isolated by screening screening a cDNA library with a tyrosine kinase domain probe from the c-fms gene (Firmbach-Kraft, I., et al., Oncogene 5:1329-1336 (1990)). The family is characterized by the presence of two kinase domains, one of which is a carboxyl domain that has all the hallmarks of protein kinases. The second domain is immediately amino terminal and bears all the hallmarks of a protein kinase but differs significantly from both the protein tyrosine and serine/threonine kinases. Amino terminal to the kinase domains, there are no SH2 and SH3 domains that characterize most of the non-receptor tyrosine kinases. However, there is extensive similarity in this region among the Jak family members and a number of homology domains have been defined (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)).

Detailed Description Text (189):
Growth hormone receptor (GHR) forms a complex with a tyrosine kinase, suggesting involvement of a ligand-activated tyrosine kinase in intracellular signaling by growth hormone (GH). Here we identify Jak2, a nonreceptor tyrosine kinase, as a GHR-associated tyrosine kinase. Immunological approaches were used to establish GH-dependent complex formation between Jak2 and GHR, activation of Jak2 tyrosine kinase activity, and tyrosyl phosphorylation of both Jak2 and GHR. The Jak2-GHR and Jak2-erythropoietin receptor interactions described here and in the accompanying Example 2 provide a molecular basis for the role of tyrosyl phosphorylation in physiological responses to these ligands, thus evidencing shared signaling mechanism among members of the cytokine/hematopoietin receptor family.

Detailed Description Text (191):
Although the ability of growth hormone (GH) to promote growth and regulate metabolism has been known for many years (Cheek, D. B. and Hill, D. E., "Effect of growth hormone on cell and somatic growth," in E. Knobli and W. H. Sawyer, eds., Handbook of Physiology, Vol. 4:159-185, Washington, D.C. (1974); Davidson, M. B., Rev. 8:115-131 (1987)), the molecular mechanism by which GH binding to its receptor elicits its diverse responses has remained an enigma. New insight into GH signaling mechanisms was recently provided by the demonstration that a tyrosine kinase activity is present in a complex with GH receptor (GHR) prepared from GH-treated fibroblasts (Carter-Su., C. et al., J. Biol. Chem. 264:18654-18661 (1989); Stred, S. E. et al., Endocrinol. 130:1626-1636 (1992); Wang, X. et al., J. Biol. Chem. 267:17390-17396 (1992)). Additional studies in 3T3-F442A cells showing rapid GH-dependent tyrosyl phosphorylation of multiple proteins, tyrosyl phosphorylation of microtubule-associated protein kinases, and stimulation of microtubule-associated protein kinase activity, as well as the inhibition of these actions by inhibitors of the GHR-associated tyrosine kinase (Campbell, G. S. et al., J. Biol. Chem. 268:7427-7434 (1993)), suggest a central role for a GHR-associated tyrosine kinase in signaling by GH. Recently, a nonreceptor tyrosyl phosphorylated 122 kd protein was identified in a kinase-active GH-GHR preparation (Wang, X. et al., J. Biol. Chem. 268:3573-3579 (1993)). Since autophosphorylation is often a manifestation of an activated kinase, it was hypothesized that this 121 kd phosphoprotein is the GHR-associated kinase.

Other Reference Publication (9):
Firmbach-Kraft, I. et al., "tyk2, prototype of a novel class of non-receptor tyrosine kinase genes," Oncogene 5:1329-1336 (3 May 1990).

☐ 11. Document ID: US 5621075 A

L10: Entry 11 of 12 File: USPT Apr 15, 1997

DOCUMENT-IDENTIFIER: US 5621075 A
** See image for Certificate of Correction **
TITLE: Insulin receptor substrate

Detailed Description Text (150):
Recently, tyrosine phosphorylation sites in MTag and various growth factor receptors, in particular the PDGF and EGF receptors, have been shown to bind specifically to the src homology-2 (SH2) domain in certain signal transduction proteins, including phosphoinositide-specific phospholipase C (PLC 1), GTPase activating protein (GAP), phosphatidyl inositol 3-kinase (PtdIns 3'-kinase) and p74.sup.raf , Anderson et al., 1990, Nature, 250,979-982, hereby incorporated by reference. The SH2 domain was first identified in nonreceptor protein tyrosine kinases like Src and Fps, by its apparent ability to interact with the kinase domain and phosphorylated substratesy. Several motifs are highly conserved within the SH2 domain which typically begins with the sequence W-(Y/F)-(H/F)-G-K. Bacterially expressed SH2 domains from PLC 1 or GAP immobilized on Sepharose precipitate the PDGF and EGF receptor, suggesting that ligand-stimulated tyrosine phosphorylation may regulate the interaction between the receptor and cellular protein. Thus, tyrosine phosphorylation enable certain proteins to bind to cellular protein containing SH2 domains and potentially altering their activity.

Other Reference Publication (24):
Maegawa et al., "Insulin Receptors with Defective Tyrosine Kinase Inhibit Normal Receptor Function at the Level of Substrate Phosphorylation", J. Biol. Chem., vol. 263, No. 25, pp. 12629-12637, Sep. 5, 1988.

☐ 12. Document ID: US 5573935 A

L10: Entry 12 of 12 File: USPT Nov 12, 1996

DOCUMENT-IDENTIFIER: US 5573935 A
TITLE: Protein tyrosine kinase A6

Brief Summary Text (5):
Tyrosine phosphorylation is an early signal transduction event which occurs after the binding of growth factors, hormones or cytokines to cell surface receptors and is a mechanism by which a number of oncogene products exert their proliferation-inducing effects (Aaronson, (1991) Science, 254: 1146-1153; Hanks et al., (1988) Science, 241: 42-52; Hunter and Cooper, (1985) Annu. Rev. Biochem., 54: 897-930). Oncogenes are mutated forms of normal genes (proto-oncogenes) which have been picked up by retroviruses. Most proto-oncogenes encode proteins mediating events by which growth factors stimulate normal cell division (Cantley et al., (1991) Cell, 64: 281-302). For example, the v-src and v-abl genes encode transforming tyrosine kinases from Rous sarcoma virus and Abelson murine leukemia virus, respectively, which are oncogenic counterparts of the corresponding normal cellular genes. These kinases may be classified as either receptor (v-erb B, v-neu) or nonreceptor (v-

src, v-abl) tyrosine kinases.

Detailed Description Text (12):
Antibodies generated against the A6 protein tyrosine kinase will be useful in the production of diagnostic kits for determining the level of the A6 kinase in both normal and neoplastic tissue to correlate its activity with cell proliferation. Antibodies against A6 will also be useful in cellular localization of the A6 kinase by immunocytochemistry. Further, these antibodies can be used for affinity chromatography to isolate large quantities of the expressed protein.

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L10: Entry 5 of 12 File: USPT Apr 3, 2001

US-PAT-NO: 6210654
DOCUMENT-IDENTIFIER: US 6210654 B1

TITLE: Jak kinases and regulation of cytokine signal transduction

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Witthuhn; Bruce A.	Memphis	TN		
Quelle; Frederick W.	Memphis	TN		
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US-CL-CURRENT: 424/9.2; 424/9.1, 424/94.1, 424/94.5, 435/21, 514/2, 514/21

CLAIMS:

What is claimed is:

1. A method for inhibiting or enhancing the biological response of a eukaryotic eukaryotic cell to a cytokine, comprising

(A) inhibiting or enhancing the tyrosine kinase activity of a murine or human Jak kinase in said eukaryotic cell, wherein said response is mediated by the activation of said Jak kinase, and wherein, when said Jak kinase is Jak2, said cytokine is other than erythropoietin (EPO) or interleukin-3 (IL-3).

2. A method according to claim 1, wherein the activity of said Jak kinase is inhibited by introducing into said eukaryotic cell effective amounts of a composition capable of inhibiting the expression of said Jak kinase in said eukaryotic cell.

3. A method according to claim 1, wherein the activity of said Jak kinase is inhibited by introducing into said eukaryotic cell effective amounts of a composition capable of inhibiting said activity.

4. A method according to claim 1, wherein said Jak kinase is murine Jak3.

5. A method for treating a disease condition in an animal caused by cytokine-induced activation of at least one member of a Jak kinase family, the method comprising, inhibiting tyrosine kinase activity of said Jak kinase in said cells, wherein when said Jak kinase is Jak2, said cytokine is not erythropoietin (EPO) or interleukin-3 (IL-3).

6. A method for identifying a composition capable of inhibiting or enhancing

the biological response of a eukaryotic cell to a cytokine whose induction of tyrosine kinase activity is mediated by the activation of a Jak kinase, comprising detecting the ability of said composition to inhibit or enhance the in vitro or in vivo kinase activity or activation of said Jak kinase, wherein, when said Jak kinase is Jak 2, said cytokine is not erythropoietin (EPO) or interleukin-3 (IL-3).

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L10: Entry 10 of 12 File: USPT Mar 17, 1998

DOCUMENT-IDENTIFIER: US 5728536 A
** See image for Certificate of Correction **
TITLE: Jak kinases and regulation of Cytokine signal transduction

Brief Summary Text (13):
Tyrosine phosphorylation has further been associated with the response to the cytokine growth hormone (GH). Studies in 3T3-F442A cells showing rapid GH-dependent tyrosyl phosphorylation of multiple proteins, tyrosyl phosphorylation of microtubule-associated protein kinases, and stimulation of microtubule-associated protein kinase activity, as well as the inhibition of these actions by inhibitors of growth hormone receptor (GHR)-associated tyrosine kinase (Campbell, G. S. et al., J. Biol. Chem. 268:7427-7434 (1993)), suggest a central role for a GHR-associated tyrosine kinase activity in signaling by GH. In addition, the presence of a tyrosine kinase activity in a complex with GH receptor (GHR) prepared from GH-treated fibroblasts has been reported (Carter-Su., C. et al., J. Biol. Chem. 264:18654-18661 (1989); Stred, S. E. et al., Endocrinol. 130:1626-1636 (1992); Wang, X. et al., J. Biol. Chem. 267:17390-17396 (1992)). More recently, a nonreceptor tyrosyl phosphorylated 122 kd protein was identified in a kinase-active GH-GHR preparation (Wang, X. et al., J. Biol. Chem. 268:3573-3579 (1993)).

Brief Summary Text (16):
Two of the other tyrosine kinases expressed in IL-3-dependent cells, Jak1 and Jak2, belong to the Jak family of kinases. The Jak (Janus kinase; alternatively referred to as just another kinase) family of kinases were initially detected in PCR amplification of tyrosine kinase domains in hematopoietic cells (Wilks, A. F., Proc. Natl. Acad. Sci. USA 86:1603-1607 (1989)). These studies identified two closely related genes (FD17 and FD22; later termed Jak2 and Jak1) from which the major PCR amplification products were derived. The complete structure of the human Jak1 gene has been reported (Wilks, A. F., et al., Mol. Cell. Biol. 11:2057-2065 (1991)) and, recently, a partial sequence of the murine Jak2 gene was published (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)). Independently a third member of the family (Tyk2) was isolated by screening a cDNA library with a tyrosine kinase domain probe from the c-fms gene (Firmbach-Kraft, I., et al., Oncogene 5:1329-1336 (1990)). The family is characterized by the presence of two kinase domains, one of which is a carboxyl domain that has all the hallmarks of protein kinases. The second domain is immediately amino terminal and bears all the hallmarks of a protein kinase but differs significantly from both the protein tyrosine and serine/threonine kinases. Amino terminal to the kinase domains, there are no SH2 and SH3 domains that characterize most of the non-receptor tyrosine kinases. However, there is extensive similarity in this region among the Jak family members and a number of homology domains have been defined (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)).

Detailed Description Text (31):
In those situations where the biological response of a cell to a cytokine is deficient due to insufficient amounts of a Jak kinase, the present invention provides for enhancing this response by increasing the levels of the Jak kinase in the cell (see Example 4). This situation could be due to mutations which reduce the amount of the Jak kinase produced by the cell to sub-normal levels. This situation

could also be due to mutations which reduce the rate or degree of cytokine-induced Jak activation such that the level of Jak kinase produced by the cell does not provide sufficient levels of activated Jak kinase following cytokine induction.

Detailed Description Text (72):
The Jak (Janus kinase; alternatively referred to as just another kinase) family of kinases was initially detected in PCR amplification of tyrosine kinase domains in hematopoietic cells (Wilks, A. F., Proc. Natl. Acad. Sci. USA 86:1603-1607 (1989)). These studies identified two closely related genes (FD17 and FD22; later termed Jak2 and Jak1) from which the major PCR amplification products were derived. The complete structure of the human Jak1 gene has been reported (Wilks, A. F., et al., Mol. Cell. Biol. 11:2057-2065 (1991)) and, recently, a partial sequence of the murine Jak2 gene was published (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)). Independently a third member of the family (Tyk2) was isolated by screening a cDNA library with a tyrosine kinase domain probe from the c-fms gene (Firmbach-Kraft, I., et al., Oncogene 5:1329-1336 (1990)). The family is characterized by the presence of two kinase domains, one of which is a carboxyl domain that has all the hallmarks of protein kinases. The second domain is immediately amino terminal and bears all the hallmarks of a protein kinase but differs significantly from both the protein tyrosine and serine/threonine kinases. Amino terminal to the kinase domains, there are no SH2 and SH3 domains that characterize most of the non-receptor tyrosine kinases. However, there is extensive similarity in this region among the Jak family members and a number of homology domains have been defined (Harpur, A. G., et al., Oncogene 7:1347-1353 (1992)).

Detailed Description Text (189):
Growth hormone receptor (GHR) forms a complex with a tyrosine kinase, suggesting involvement of a ligand-activated tyrosine kinase in intracellular signaling by growth hormone (GH). Here we identify Jak2, a nonreceptor tyrosine kinase, as a GHR-associated tyrosine kinase. Immunological approaches were used to establish GH-dependent complex formation between Jak2 and GHR, activation of Jak2 tyrosine kinase activity, and tyrosyl phosphorylation of both Jak2 and GHR. The Jak2-GHR and Jak2-erythropoietin receptor interactions described here and in the accompanying Example 2 provide a molecular basis for the role of tyrosyl phosphorylation in physiological responses to these ligands, thus evidencing shared signaling mechanism among members of the cytokine/hematopoietin receptor family.

Detailed Description Text (191):
Although the ability of growth hormone (GH) to promote growth and regulate metabolism has been known for many years (Cheek, D. B. and Hill, D. E., "Effect of growth hormone on cell and somatic growth," in E. Knobli and W. H. Sawyer, eds., Handbook of Physiology, Vol. 4:159-185, Washington, D.C. (1974); Davidson, M. B., Rev. 8:115-131 (1987)), the molecular mechanism by which GH binding to its receptor elicits its diverse responses has remained an enigma. New insight into GH signaling mechanisms was recently provided by the demonstration that a tyrosine kinase activity is present in a complex with GH receptor (GHR) prepared from GH-treated fibroblasts (Carter-Su., C. et al., J. Biol. Chem. 264:18654-18661 (1989); Stred, S. E. et al., Endocrinol. 130:1626-1636 (1992); Wang, X. et al., J. Biol. Chem. 267:17390-17396 (1992)). Additional studies in 3T3-F442A cells showing rapid GH-dependent tyrosyl phosphorylation of multiple proteins, tyrosyl phosphorylation of microtubule-associated protein kinases, and stimulation of microtubule-associated protein kinase activity, as well as the inhibition of these actions by inhibitors of the GHR-associated tyrosine kinase (Campbell, G. S. et al., J. Biol. Chem. 268:7427-7434 (1993)), suggest a central role for a GHR-associated tyrosine kinase in signaling by GH. Recently, a nonreceptor tyrosyl phosphorylated 122 kd protein was identified in a kinase-active GH-GHR preparation (Wang, X. et al., J. Biol. Chem. 268:3573-3579 (1993)). Since autophosphorylation is often a manifestation of an activated kinase, it was hypothesized that this 121 kd phosphoprotein is the GHR-associated kinase.

Other Reference Publication (9):
Firmbach-Kraft, I. et al., "tyk2, prototype of a novel class of non-receptor tyrosine kinase genes," Oncogene 5:1329-1336 (3 May 1990).

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DOCUMENT-IDENTIFIER: US 6569865 B2
** See image for Certificate of Correction **
TITLE: Spiro 1-azabicyclo[2.2.2]octane-3,2'(3'h)-furo[2,3-b]pyridine

CLAIMS:

14. A method of using a compound according to claim 12, in a screen for the discovery of novel medicinal compounds which bind to and modulate the activity, via agonism, partial agonism, or antagonism, of the .alpha..sub.7 nicotinic acetylcholine receptor.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 8. Document ID: US 6485929 B1

L25: Entry 8 of 33 File: USPT Nov 26, 2002

DOCUMENT-IDENTIFIER: US 6485929 B1
TITLE: Method for inhibiting CD95-independent apoptosis in AIDS

CLAIMS:

3. The method according to claim 1, wherein the competing factor is an anti-CXCR4 antibody.

4. The method according to any one of claims 1 to 3, wherein (a) is blocked by a natural ligand of CD4 and/or a natural ligand of CXCR4.

7. A system for identifying substances suitable to inhibit CD95-independent apoptosis, comprising CD4.sup.+ and/or CXCR4.sup.+ cells, HIV-1 gp120, and a factor competing with HIV-1 gp120 for the bonding to CD4.sup.+ and/or CXCR4.sup.+.

8. The system according to claim 7, wherein the inhibitory effect of said factor is determined on the bonding of HIV-1 gp120 to receptors CD4 and/or CXCR4 or on the signal path induced by this bonding.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 9. Document ID: US 6472218 B1

L25: Entry 9 of 33 File: USPT Oct 29, 2002

DOCUMENT-IDENTIFIER: US 6472218 B1
TITLE: Systems and methods for rapidly identifying useful chemicals in liquid samples

CLAIMS:

1. A method of modulating the activity of a selected molecular targets with a selected chemical compound, comprising: storing a set of chemical compounds in addressable wells in a chemical library; programming a chemical storage and retrieval module containing said chemical compounds for the selection of a subset of said chemical compounds; removing said subset of said addressable chemical wells from sad chemical storage module with an automated robotic retriever; routing said selected subset of addressable chemical wells away from said chemical storage and retrieval module and toward a liquid processing apparatus in a transport pathway; retrieving said programmed subset of said chemical compounds with said liquid processing apparatus by retrieving liquid samples from less than all of said removed subset of addressable chemical wells; testing, in said liquid processing apparatus, said selected set of chemical compounds for modulating activity against one or more selected molecular targets; selecting a chemical compound from among said selected set of chemical compounds that modulates that activity of at least one or more selected molecular targets; and contacting said selected chemical compound with said selected molecular target such that the activity of said selected molecular target is modulated.

3. The method of claim 1, wherein said chemical compound has modulating activity with respect to said molecular target selected from the group consisting of ion channels, N-methyl-D-aspartate receptor, kainite receptors, AMPA receptor, GABA receptors, nicotinic acetylcholine receptors, excitatory amino acid receptors, soluble proteins, membrane proteins, hormone receptors, transcription factors, proteases, kinases, and phosphatases.

4. The method of claim 1, wherein said testing comprises testing at a rate of at least 50,000 chemical compounds in 24 hours.

5. The method of claim 1, wherein said testing comprises testing at a rate of at least 100,000 chemical compounds in 24 hours.

6. The method of claim 1, wherein said testing comprises testing at a rate of at least 300,000 compounds in 24 hours.

7. The method of claim 1, wherein said testing comprises testing at a rate of at least 500,000 chemical compounds in 24 hours.

8. The method of claim 1, wherein said testing comprises testing at a rate of at least 1,000,000 chemical compounds in 24 hours.

9. The method of claim 1, wherein said testing comprises a cell based assay.

10. The method of claim 1, wherein said testing comprises a biochemical assay.

☐ 10. Document ID: US 6455754 B1

L25: Entry 10 of 33 File: USPT Sep 24, 2002

DOCUMENT-IDENTIFIER: US 6455754 B1

TITLE: GENOMIC DNA FRAGMENTS CONTAINING REGULATORY AND CODING SEQUENCES FOR THE .beta.2-SUBUNIT OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR AND TRANSGENIC ANIMALS MADE USING THESE FRAGMENTS OR MUTATED FRAGMENTS

CLAIMS:

1. A method of screening for a compound that detectably affects activity of a neuronal nicotinic acetylcholine receptor, comprising: (a) introducing the compound into a transgenic mouse all of whose somatic cells and germ cells contain a homozygous disruption of the endogenous .beta.2-subunit of the neuronal nicotinic acetylcholine receptor, wherein the homozygous disruption results in the absence of expression of the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and a lack of inward current activity in anterior thalamic neurons in response to a nicotinic acetylcholine receptor agonist in the mouse; and (b) selecting the compound that detectably affects activity of the neuronal nicotinic acetylcholine receptor.

2. A method of screening for a compound that detectably affects activity of a neuronal nicotinic acetylcholine receptor, comprising: (a) contacting the compound with a neuronal cell line, wherein the neuronal cell line is derived from a transgenic mouse all of whose somatic cells and germ cells contain a homozygous disruption of the endogenous .beta.2-subunit of the neuronal nicotinic acetylcholine receptor, wherein the homozygous disruption results in the absence of expression of the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and a lack of inward current activity in anterior thalamic neurons in response to a nicotinic acetylcholine receptor agonist in the mouse; and (b) selecting the compound that detectably affects activity of the neuronal nicotinic acetylcholine receptor.

3. A method of screening for a compound that detectably affects activity of a neuronal nicotinic acetylcholine receptor, comprising: (a) introducing the compound into a first transgenic mouse, wherein the first transgenic mouse is generated by providing a second transgenic mouse all of whose somatic cells and germ cells contain a homozygous disruption of the endogenous .beta.2-subunit of the neuronal nicotinic acetylcholine receptor, wherein the homozygous disruption results in the absence of expression of the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and a lack of inward current activity in anterior thalamic neurons in response to a nicotinic acetylcholine receptor agonist in the mouse, and crossing the second transgenic mouse with a mouse to generate the first transgenic mouse, wherein nicotine binding in the brain of the first transgenic mouse is reduced by at least approximately 50% as compared to a wild-type mouse; and (b) selecting the compound that detectably affects activity of the neuronal nicotinic acetylcholine receptor.

7. A method of screening for a compound that modulates activity of a promoter sequence of the .beta.-2 subunit of a neuronal nicotinic acetylcholine receptor, comprising: (a) contacting the compound with a cell line, wherein the cell line is a muscle cell line or a neuronal cell line and comprises in its genome a promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor operatively linked to a heterologous sequence encoding a polypeptide; and (b) selecting the compound that modulates the activity of the promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor; wherein the promoter sequence is selected from the group consisting of: (A) the nucleic acid sequence from about nucleotide -1125 to about nucleotide +38 as set forth in FIG. 1 (SEQ ID NO. 22); and (B) a sequence having promoter activity, which hybridizes to DNA complementary to the sequence (A) under stringent conditions, wherein the stringent conditions comprise a temperature of about 65.degree. C. and an SSC buffer concentration of about 0.1.times.SSC.

10. A method of screening for a compound that increases or decreases activity of a promoter sequence of the .beta.-2 subunit of a neuronal nicotinic acetylcholine receptor, comprising: (a) contacting the compound with a cell line, wherein the cell line is a muscle cell line or a neuronal cell line and comprises a promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor operatively linked to a heterologous sequence encoding a polypeptide, wherein the promoter sequence comprises the nucleic acid sequence from about nucleotide -1125 to about nucleotide +38 as set forth in FIG. 1 (SEQ ID NO. 22); (b) measuring directly or indirectly the expression of the polypeptide; and (c) selecting the compound that increases or decreases expression of the polypeptide, wherein an increase or decrease in polypeptide expression correlates with an increase or decrease in activity of the promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor.

11. A method of screening for a compound that increases or decreases activity of a promoter sequence of the .beta.-2 subunit of a neuronal nicotinic acetylcholine receptor, comprising: (a) contacting the compound with a cell line, wherein the cell line is a muscle cell line or a neuronal cell line and comprises a promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor operatively linked to a heterologous sequence encoding a polypeptide, wherein the promoter sequence is selected from the group consisting of a nucleic acid sequence from about nucleotide -968 to about nucleotide +38, a nucleic acid sequence from about nucleotide -824 to about nucleotide +38, and a nucleic acid sequence from about nucleotide -245 to about nucleotide +38, as set forth in FIG. 1 (SEQ ID NO. 22); (b) measuring directly or indirectly the expression of the polypeptide; and (c) (c) selecting the compound that increases or decreases expression of the polypeptide, wherein an increase or decrease in polypeptide expression correlates with an increase or decrease in activity of the promoter sequence of the .beta.2 subunit of the neuronal nicotinic acetylcholine receptor.

12. A method of screening for a compound that increases or decreases activity of a promoter sequence of the .beta.-2 subunit of a neuronal nicotinic acetylcholine receptor, comprising: (a) contacting the compound with a cell line, wherein the cell line is a muscle cell line or a neuronal cell line and comprises a promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor, and wherein the promoter sequence is obtained by the process comprising (i) hybridizing a fragment of a .beta.2-subunit of the neuronal nicotinic acetylcholine receptor gene from a first species with the genomic DNA of a second species under stringent conditions, wherein the stringent conditions comprise a temperature of about 65.degree. C. and an SSC buffer concentration of about 0.1.times.SSC; and (ii) isolating the promoter sequence of the second species from the hybridized sequences; wherein the promoter sequence of the .beta.2 subunit of the neuronal nicotinic acetylcholine receptor is operatively linked to a heterologous sequence encoding a polypeptide; (b) measuring directly or indirectly the expression of the polypeptide; and (c) selecting the compound that increases or decreases expression of the polypeptide, wherein an increase or decrease in polypeptide expression correlates with an increase or decrease in activity of the promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor.

13. A method of screening for a compound that increases or decreases activity of a promoter sequence of the .beta.-2 subunit of a neuronal nicotinic acetylcholine receptor, comprising: (a) contacting the compound with a cell line, wherein the cell line is a muscle cell line or a neuronal cell line and comprises a promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor operatively linked to a heterologous sequence encoding a polypeptide; (b) measuring directly or indirectly the expression of the polypeptide; and (c) selecting the compound that increases or decreases expression of the polypeptide, wherein an increase or decrease in polypeptide expression correlates with an increase or decrease in activity of the promoter sequence of the .beta.-2 subunit of the neuronal nicotinic acetylcholine receptor, wherein the promoter sequence is

selected from the group consisting of: (A) the nucleic acid sequence from about nucleotide -1125 to about nucleotide +38 as set forth in FIG. 1 (SEQ ID NO. 22); and and (B) a sequence having promoter activity, which hybridizes to DNA complementary to the sequence (A) under stringent conditions, wherein the stringent conditions comprise a temperature of about 65.degree. C. and an SSC buffer concentration of about 0.1.times.SSC.

☐ 11. Document ID: US 6451598 B1

L25: Entry 11 of 33 File: USPT Sep 17, 2002

DOCUMENT-IDENTIFIER: US 6451598 B1
TITLE: Cell fusion assays for the identification of antiviral compounds, and systems systems and kits for practicing the same

CLAIMS:

1. A method of assaying whether a candidate agent inhibits CD4/CCR5-mediated fusion of a human immunodeficiency virus (HIV) virion and its target cell, said method comprising: (a) combining under cell fusion conditions said candidate agent with: (i) a first eukaryotic cell that stably displays an envelope protein of said HIV virion on its surface; and (ii) a second eukaryotic cell that stably displays both a CD4 receptor and a CCR5 receptor of said target cell for said envelope protein on its surface; wherein said first and second cells further comprise a stably-expressed two component signal producing system that provides a detectable signal upon fusion of said first and second cells; (b) identifying the presence or absence of said detectable signal; and (c) deriving the inhibitory activity of said candidate agent from the presence or absence of said detectable signal, wherein inhibitory activity indicates that the candidate agent inhibits CD4/CCD5-mediated fusion of and HIV virion and its target cell.

6. The method according to claim 5, wherein said identifying step (b) comprises: (i) lysing said contacted cells; (ii) contacting said lysed cells with substrate that is converted by said reporter enzyme into a detectable product if cell fusion has occurred; (iii) detecting the presence or absence of said detectable product.

10. The method according to claim 1, wherein said method is a high throughput screening method that simultaneously assays a plurality of candidate inhibitory agents for cell fusion inhibitory activity.

11. A method of assaying whether a candidate agent inhibits CD4/CR5-mediated fusion of a human immunodeficiency virus (HIV) virion and its target cell, said method comprising: (a) combining under cell fusion conditions said candidate agent with: (i) a Chinese hamster ovary cell having a coding sequence for an envelope protein of said HIV virion stably integrated into its genome and that stably displays an envelope protein of said enveloped virion on its surface; and (ii) a second eukaryotic cell having a coding sequence for both a CD4 receptor and a CCR5 receptor of said target cell for said envelope protein integrated into its genome and that stably displays said receptors on its surface; wherein said first and second cells further comprise a Tat reporter system that provides a detectable fluorescent signal upon fusion of said first and second cells; (b) identifying the

presence or absence of said fluorescent detectable signal by: (i) lysing said contacted cells; (ii) contacting said lysed cells with substrate that is converted into a fluorescent detectable product if cell fusion has occurred; and (iii) detecting the presence or absence of said detectable product; and (c) deriving the inhibitory activity of said candidate agent from the presence or absence of said detectable fluorescent signal, wherein inhibitory activity indicates that the candidate agent inhibits CD4/CCD5-mediated fusion of and HIV virion and its target cell.

14. The method according to claim 11, wherein said method is a high throughput screening method that simultaneously assays a plurality of candidate inhibitory agents for cell fusion inhibitory activity.

☐ 12. Document ID: US 6440681 B1

L25: Entry 12 of 33 File: USPT Aug 27, 2002

DOCUMENT-IDENTIFIER: US 6440681 B1
TITLE: Methods for identifying agonists and antagonists for human neuronal nicotinic acetylcholine receptors

CLAIMS:

1. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells are produced by transfection with nucleic acid encoding at least one human nNAChR subunit; wherein the at least one subunit is an .alpha..sub.2 subunit; the recombinant cells express an nNAChR comprising at least one human subunit encoded by the transfected nucleic acid; and the expressed nNAChR comprises at least one nNAChR .alpha. subunit; and b) measuring ion flux, the electrophysiological response response of the cells, or binding of the test compound to the nNAChR, whereby agonist or antagonists of the nNAChR are identified, wherein; the .alpha..sub.2 subunit comprises a sequence of amino acids having more than 95% identity with amino acid sequence set forth in SEQ ID No.2; or the .alpha..sub.2 subunit is encoded by a sequence of nucleotides that hybridizes under conditions of high stringency with the complement of nucleotides 166-1755 set forth in SEQ ID No. 1 or shares at least about 90% identity to nucleotides 166-1755 in SEQ ID No: 1; or the .alpha..sub.2 subunit is encoded by the nucleotides of 166-1755 of SEQ ID No. 1.

28. The method of claim 1, wherein which is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

29. The method of claim 1, wherein the cells further comprise nucleic acid encoding

a .beta..sub.2 subunit that comprises amino acids encoded by the DNA in a plasmid having all of the identifying characteristics of the plasmid depositeds ATCC No. 68279.

37. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid wherein the at least one subunit is an .alpha..sub.2 subunit; and b) measuring ion flux, the electrophysiological response of the cells, or binding of the test compound to the nNAChR, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein said .alpha..sub.2 subunit comprises the sequence of amino acids set forth in SEQ ID NO:2.

38. The method of claim 37, wherein the .alpha..sub.2 subunit comprises amino acids encoded by the DNA in a plasmid having all of the identifying characteristics of the plasmid deposited as ATCC No. 68277.

47. The method of claim 37, in which an agonist is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

51. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.3 subunit; and b) measuring ion flux, the electrophysiological response of the cells, or interaction of the test compound with the nNAChRs, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein: the .alpha..sub.3 subunit comprises a sequence of amino acids encoded by the .alpha..sub.3 -encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR.alpha.3 deposited under ATCC Accession No. 68278; or comprises the sequence of amino acids set forth in SEQ ID NO:4.

53. The method of claim 51, wherein the .alpha..sub.3 subunit comprises amino acids encoded by the DNA in a plasmid having all of the identifying characteristics of the plasmid deposited as ATCC No. 68278.

62. The method of claim 51, in which an agonist is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

65. The method according to claim 51, wherein the .alpha..sub.3 subunit comprises a sequence of amino acids encoded by the .alpha..sub.3 encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR .alpha.3 deposited under ATCC Accession No. 68278.

66. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and b) measuring ion flux, the electrophysiological response of the cells, or interaction of the test compound with the nNAChRs, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein: the .alpha..sub.4 the .alpha..sub.4 subunit is encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to nucleic acid that encodes the sequence of amino acids set forth in SEQ ID No:6 or the .alpha..sub.4 subunit comprises the sequence of amino acids encoded by .alpha.4 subunit encoding nucleic acid in clone HnAChR .alpha.4.2, deposited under ATCC Accession No. 69239; or the .alpha..sub.4 subunit comprises the sequence of amino acids encoded by .alpha.4 subunit encoding nucleic acid in clone HnAChR .alpha.4.1, deposited under ATCC Accession No. 69152; the .alpha..sub.4 subunit is encoded by nucleic acid that hybridizes under conditions of high stringency to nucleotides 184-2067 of SEQ ID No. 5; or the .alpha..sub.4 subunit is encoded by nucleic acid that hybridizes under conditions of high stringency to the entire .alpha.4 encoding insert of clone HnAChR .alpha.4.2deposited under ATCC Accession No. 69239.

76. The method of claim 66, in which an agonist is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

85. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.6 subunit; and b) measuring ion flux, the electrophysiological response of the cells or interaction of the test compound with the nNAChRs, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein: the .alpha..sub.6 subunit comprises a sequence of amino acids encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to the coding sequence in the sequence of nucleotides set forth in SEQ ID No. 9, or the .alpha..sub.6 subunit comprises the sequence of amino acids sequence set forth in SEQ ID No. 10.

95. The method of claim 85, which an agonist is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

101. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nHAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous

nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.7 subunit and b) measuring ion flux, the electrophysiological response of the cells, or interaction of the test compound with the nNAChRs, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein: the .alpha..sub.7 subunit comprises a sequence of amino acids encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to the coding sequence in the sequence of nucleotides set forth in SEQ ID No. 11, or the .alpha..sub.7 subunit comprises the sequence of amino acid sequence set forth in SEQ ID No. 12.

111. The method of claim 101, which an agonist is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

118. A method for identifying compound that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.5 subunit; and b) measuring ion flux, the electrophysiological response of the cells, or binding of the test compound to the nNAChR, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein: the .alpha..sub.5 subunit comprises a sequence of amino acids encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to the coding sequence in the sequence of nucleotides set forth in SEQ ID No. 7, or the .alpha..sub.5 subunit comprises the sequence of amino acid sequence set forth in SEQ ID No. 8.

127. The method of claim 118, which an agonist is detected, wherein: the recombinant cells further comprise a DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; the reporter gene encodes a detectable gene product, wherein wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and the interaction of the test compound with the nNAChRs is measured by detecting the gene product encoded by the reporter gene.

135. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; the recombinant cells express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; the heterologous nucleic acid encodes a .beta..sub.2 subunit of a human nNAChR; and the expressed nNAChR comprises at least one nNAChR .alpha. subunit and the .beta..sub.2 subunit; and (b) measuring ion flux, the electrophysiological response of the cells, or interaction of the test compound to the nNAChR, whereby compounds that are agonists or antagonists of the nNAChR are identified, wherein the .beta..sub.2 subunit comprises the sequence of amino acids set forth in SEQ ID No. 14.

141. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; and

the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.3 subunit, wherein: the .alpha..sub.3 subunit comprises a sequence of of amino acids encoded by the .alpha..sub.3 -encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR .alpha..sub.3 HnAChR .alpha..sub.3 deposited under ATCC Accession No. 68278; or comprises the sequence of amino acids set forth in SEQ ID No. 4; and the recombinant cells further comprise DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element, wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; receptor; the reporter gene encodes a detectable gene product, wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and b) measuring transcription of the reporter gene by detecting the gene product, whereby compounds that are agonists or antagonists of the nNAChR are identified.

143. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acids is an .alpha..sub.4 subunit, wherein: the .alpha..sub.4 subunit is encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to a nucleic acid that encodes the sequence of amino acids set forth in SEQ ID No: 6; the .alpha..sub.4 subunit is encoded by nucleic acid that hybridizes under conditions of high stringency to the entire .alpha..sub.4 encoding insert of clone HnAChR.alpha.4.2, deposited under ATCC Accession No. 69239; the .alpha..sub.4 subunit comprises the sequence of amino acids encoded by .alpha..sub.4 subunit encoding nucleic acid in cline HnAChR .alpha.4.1, deposited under ATCC Accession No. 69152; the .alpha..sub.4 subunit is encoded by the nucleic acid that hybridizes along it full length under conditions of high stringency to the entire length of nucleotides 184-2067 of SEQ ID No. 5; or the .alpha..sub.4 subunit is encoded by nucleic acid that hybridizes under conditions of high stringency to the entire .alpha..sub.4 encoding insert of clone HnAChR.alpha.4.2, deposited under ATCC Accession No. 69239; and the recombinant cells further comprise DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element, wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; and the reporter gene encodes a detectable gene product, wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and b) measuring transcription of the reporter gene by detecting the gene product, whereby compounds that are agonists or antagonists of the nNAChR are identified.

150. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.6 subunit, wherein: the .alpha..sub.6 subunit comprises a sequence of amino acids encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to the coding sequence in the sequence of nucleotides set forth in SEQ ID No. 9, or the .alpha..sub.6 subunit comprises the sequence of amino acid sequence set forth in SEQ ID No. 10; and the recombinant cells further comprise DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element, wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; and the reporter gene encodes a detectable gene product, wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and b) measuring transcription of the reporter gene by detecting the

gene product, whereby compounds that are agonists or antagonists of the nNAChR are identified.

154. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.7 subunit, wherein: the .alpha..sub.7 subunit comprises a sequence of amino acids encoded by nucleic acid that hybridizes along its full length under conditions of high stringency to the coding sequence in the sequence of nucleotides set forth in SEQ ID No. 11, or the .alpha..sub.7 subunit comprises the sequence of amino acid sequence set forth in SEQ ID No. 12; and the recombinant cells further comprise DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element, wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; and the reporter gene encodes a detectable gene product, wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and b) measuring transcription of the reporter gene by detecting the gene product, whereby compounds that are agonists or antagonists of the nNAChR are identified.

159. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.5 subunit, wherein: the .alpha..sub.5 subunit comprises a sequence of amino acids encoded by a nucleic acid that hybridizes along its full length under conditions of high stringency to the coding sequence in the sequence of nucleotides set forth in SEQ ID No. 7, or the .alpha..sub.5 subunit comprises the sequence of amino acid sequence set forth in SEQ ID No. 8; and the recombinant cells further comprise DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element, wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic acetylcholine receptor; and the reporter gene encodes a detectable gene product, wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and b) measuring transcription of the reporter gene by detecting the gene product, whereby compounds that are agonists or antagonists of the nNAChR are identified.

165. A method for identifying compounds that are antagonists or agonists of human neuronal nicotinic acetylcholine receptors (nNAChRs), said method comprising: a) contacting recombinant cells with a test compound, wherein: the recombinant cells comprise heterologous nucleic acid encoding at least one human nNAChR subunit; express an nNAChR comprising at least one human subunit encoded by the heterologous nucleic acid; and the at least one human nNAChR subunit encoded by the heterologous nucleic acid is an .alpha..sub.2 subunit, wherein: the .alpha..sub.2 subunit comprises a sequence of amino acids having more than 95% identity with amino acid sequence set forth in SEQ ID No. 2; or the .alpha..sub.2 subunit is encoded by a sequence of nucleotides that hybridizes under conditions of high stringency with the complement of nucleotides 166-1755 set forth in SEQ ID No. 1 or shares at least about 90% identity to nucleotides 166-1755 in SEQ ID No:1; or .alpha..sub.2 subunit comprises the sequence of amino acids set forth in SEQ ID NO:2; or the .alpha..sub.2 subunit is encoded by the nucleotides 166-1755 of SEQ ID No. 1; and the recombinant cells further comprise DNA encoding a reporter gene operatively linked to DNA encoding a transcriptional control element, wherein the activity of the transcriptional control element is regulated by the human neuronal nicotinic

acetylcholine receptor; and the reporter gene encodes a detectable gene product, wherein the detectable product is selected from the group consisting of mRNA and a polypeptide; and b) measuring transcription of the reporter gene by detecting the gene product, whereby compounds that are agonists or antagonists of the nNAChR are identified.

☐ 13. Document ID: US 6383746 B1

L25: Entry 13 of 33 File: USPT May 7, 2002

DOCUMENT-IDENTIFIER: US 6383746 B1
TITLE: Functional promoter for CCR5

CLAIMS:

1. An isolated functional CCR5 regulatory sequence consisting of nucleotide 36 to nucleotide 1006 of SEQ ID NO: 1.

2. An isolated CCR5 regulatory nucleic acid molecule consisting of nucleotides 36 to to 1006 of SEQ ID NO:1, and functional portions of said nucleic acid molecule, wherein the functional portion comprises nucleotide 927 to nucleotide 1006 of SEQ ID NO: 1.

3. An isolated CCR5 regulatory nucleic acid molecule consisting of nucleotides 36 to to 1006 of SEQ ID NO:1, and functional portions of said nucleic acid molecule, wherein the functional portion comprises nucleotide 521 to nucleotide 763 of SEQ ID NO: 1.

4. An isolated CCR5 regulatory nucleic acid molecule consisting of nucleotides 36 to to 1006 of SEQ ID NO:1, and functional portions of said nucleic acid molecule, wherein the functional portion comprises nucleotide 763 to nucleotide 926 of SEQ ID NO:1.

5. The CCR5 regulatory sequence of claim 1, operably linked to a nucleic acid sequence encoding a heterologous protein.

6. The CCR5 regulatory sequence of claim 2, operably linked to a nucleic acid sequence encoding a heterologous protein.

7. The CCR5 regulatory sequence of claim 3, operably linked to a nucleic acid sequence encoding a heterologous protein.

8. The CCR5 regulatory sequence of claim 4, operably linked to a nucleic acid sequence encoding a heterologous protein.

17. An antisense nucleic acid sequence, comprising an oligonucleotide or oligonucleotide analog which binds to a CCR5 regulatory sequence and reduces the activity thereof, wherein said nucleic acid is a triple-helix forming agent that binds to a sequence of SEQ ID NO:1 selected from the group consisting of nucleotides 927 to 1006 of SEQ ID NO: 1 and nucleotides 521 to 763 of SEQ ID NO: 1.

19. A method for identifying a composition which suppresses the expression of CCR5, said method comprising:

(a) incubating the composition and the CCR5 regulatory sequence of claim 1 operably linked to a detectable reporter gene under conditions sufficient to allow the composition and CCR5 regulatory sequence to interact; and

(b) identifying a composition which suppresses CCR5 regulatory region activity by selecting the composition which results in a reduction in the level of expression of the reporter gene operably linked to the CCR5 regulatory sequence in comparison to the level of reporter gene expression in the absence of said composition.

20. A method for identifying a composition which suppresses the expression of CCR5, said method comprising:

(a) incubating the composition and the CCR5 regulatory sequence of claim 2 operably linked to a detectable reporter gene under conditions sufficient to allow the composition and CCR5 regulatory sequence to interact; and

(b) identifying a composition which suppresses CCR5 regulatory region activity by selecting the composition which results in a reduction in the level of expression of the reporter gene operably linked to the CCR5 regulatory sequence in comparison to the level of reporter gene expression in the absence of said composition.

21. A method for identifying a composition which suppresses the expression of CCR5, said method comprising:

(a) incubating the composition and the CCR5 regulatory sequence of claim 3 operably linked to a detectable reporter gene under conditions sufficient to allow the composition and CCR5 regulatory sequence to interact; and

(b) identifying a composition which suppresses CCR5 regulatory region activity by selecting the composition which results in a reduction in the level of expression of the reporter gene operably linked to the CCR5 regulatory sequence in comparison to the level of reporter gene expression in the absence of said composition.

22. A method for identifying a composition which suppresses the expression of CCR5, said method comprising:

(a) incubating the composition and the CCR5 regulatory sequence of claim 4 operably linked to a detectable reporter gene under conditions sufficient to allow the composition and CCR5 regulatory sequence to interact; and

(b) identifying a composition which suppresses CCR5 regulatory region activity by selecting the composition which results in a reduction in the level of expression of the reporter gene operably linked to the CCR5 regulatory sequence in comparison to the level of reporter gene expression in the absence of said composition.

☐ 14. Document ID: US 6365356 B1

L25: Entry 14 of 33 File: USPT Apr 2, 2002

DOCUMENT-IDENTIFIER: US 6365356 B1
TITLE: Receptors that regulate cell signaling relating to chemokines

CLAIMS:

1. A method for discovering molecules that regulate cell signaling specific to the dual presence of Duffy antigen receptor for chemokines (DARC) and a chemokine receptor wherein the chemokine receptor is CXCR4, the method comprising:

providing a cell that co-expresses DARC and the chemokine receptor;

incubating the molecules with the cell;

measuring the cell signaling in the cell specific to the dual presence of DARC and the chemokine receptor; and

determining whether the cell signaling specific to te dual presence of DARC and the chemokine receptor is regulated by the molecules.

19. A method for determining a signal transduction pathway in a cell that co-expresses DARC and a chemokine receptor, wherein the chemokine receptor is CXCR4, the method comprising;

providing a cell that co-expresses DARC and the chemokine receptor;

providing a molecule known to regulate cell signaling specific to the dual presence of DARC and the chemokine receptor;

contacting the molecule with the cell;

measuring the regulation of cell signaling specific to the dual presence of DARC and and the chemokine receptor; and

determining the signal transduction pathway of the regulation in the cell.

22. A method for discovering molecules that regulate cell signaling specific to the dual presence of DARC and a chemokine receptor, wherein the chemokine receptor is CXCR4, the method comprising:

providing DARC and labeled ligands of DARC;

providing the chemokine receptor and labeled ligands of the chemokine receptor;

contacting the labeled ligands and the receptors with the molecules; and

determining whether the molecules compete successfully with the ligands, wherein successful competition indicates that the molecules regulate cell signaling specific to the dual presence of DARC and the chemokine receptor.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 15. Document ID: US 6321105 B1

L25: Entry 15 of 33 File: USPT Nov 20, 2001

DOCUMENT-IDENTIFIER: US 6321105 B1
** See image for Certificate of Correction **
TITLE: Method for diagnosing neurological, neurodegenerative and psychiatric diseases by magnetic resonance imaging using contrast agents with high magnetic susceptibility and extended plasma half life

CLAIMS:

1. A method for magnetic resonance imaging (MRI) of changes in neurotransmitter and neuroreceptor activity as a metabolic response to diagnostic challenge or therapeutic treatment in a patient with suspected or already diagnosed mental illnesses of psychiatric, neurodegenerative or neurological nature, comprising the steps of:

(a) administering to said patient a drug eliciticiting an MRI detectable hemodynamic response;

(b) administering to said patient an MRI ferromagnetic, antiferromagnetic or superparamagnetic contrast agent with high magnetic susceptibility and

(c) measuring, in a spatially and temporally resolved manner, relative Cerebral Blood Volume (rCBV) changes associated to neuronal activating using T.sub.2 - or T.sub.2 *-weighted MRI scans at the equilibrium distribution of said contrast agent.

10. The method as described in claim 9 wherein said contrast agents are used for guidance and assessment of success of therapy by assessing the effect of cholinesterase inhibitors, acetylcholine agonists or of other anti AD medications.

13. The method of claim 11 carried out by detecting rCBV changes associated with hyperactivity of postsynaptic dopamine receptors.

15. A method for measuring or depicting in a spatially and timely resolved manner the changes in regional Cerebral blood Volume (rCBV mapping) associated with changes in neurotransmitter activity exploiting the susceptibility contract effect, employing the compartmentalization of susceptibility contrast agents in the vasculature and of the constant or nearly constant concentration in blood over time comprising the steps of:

(a) administering to said patient a drug eliciticiting an MRI detectable hemodynamic response;

(b) administering to said patient an MRI ferromagnetic, antiferromagnetic or superparamagnetic contrast agent with high magnetic susceptibility and

(c) measuring, in a spatially and temporally resolved manner, relative Cerebral Blood Volume (rCBV) changes associated to neuronal activating using T.sub.2 - or T.sub.2 *weighted MRI scans at the equilibrium distribution of said contrast agent, and relating the changes in signal intensity (SI) in T=.sub.2 *- or T.sub.2 - weighted MR images to changes of rCBV.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 16. Document ID: US 6177242 B1

L25: Entry 16 of 33 File: USPT Jan 23, 2001

DOCUMENT-IDENTIFIER: US 6177242 B1
** See image for Certificate of Correction **
TITLE: Genomic DNA fragments containing regulatory and coding sequences for the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and transgenic animals made using these fragments or mutated fragments

CLAIMS:

12. A method of detecting a protein, which binds to a promoter, comprising incubating the DNA as claimed in claim 1 with a nuclear extract under conditions suitable to allow a protein in said nuclear extract to bind to said DNA to form a DNA/protein complex, and detecting said DNA/protein complex.

33. A method of detecting a protein, which binds to a promoter, comprising incubating the DNA as claimed in claim 22 with a nuclear extract under conditions suitable to allow a protein in said nuclear extract to bind to said DNA to form a DNA/protein complex, and detecting said DNA/protein complex.

42. An isolated DNA fragment having a regulatory or coding sequence of the mouse .beta.2-subunit of neuronal nicotinic acetylcholine receptor obtainable by cutting the DNA of the phage of claim 41 with restriction enzyme or mechanical shearing.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw D

☐ 17. Document ID: US 6165126 A

L25: Entry 17 of 33 File: USPT Dec 26, 2000

DOCUMENT-IDENTIFIER: US 6165126 A
TITLE: Remediation of depression through computer-implemented interactive behavioral training

CLAIMS:

16. The computer-implemented method of claim 1 wherein said computer-implemented interactive behavioral training is further directed to upregulate modulatory functions of dopamine and acetylcholine.

35. A computer-implemented method for reducing the effects of depression for a human subject, said computer-implemented method comprising:

administering, using said computer-implemented approach, a training regime including at least one behavioral test to said human subject, said training regime

being configured to substantially augment the behaviorally appropriate release of modulatory neuotransmitters associated with at least one of remediating depression and the clinical symptoms of depression;

obtaining, using said computer-implemented approach, a performance response of said human subject in said at least one behavioral test; and

altering, using said computer-implemented approach, testing parameters pertaining to to said at least one behavioral test, wherein said altering of said testing parameters facilitates substantially augmenting the behaviorally appropriate release of modulatory neuotransmitters associated with at least one of remediating depression and the clinical symptoms of depression.

☐ 18. Document ID: US 6136550 A

L25: Entry 18 of 33 File: USPT Oct 24, 2000

DOCUMENT-IDENTIFIER: US 6136550 A
TITLE: Neuronal nicotinic acetylcholihne receptor compositions

CLAIMS:

12. A method for screening drug substances to determine those drug substances which function as agonists for nicotinic acetylcholine receptors, and method comprising:

contacting a receptor with a drug substance wherein said receptor is comprised of a substantially pure neuronal nicotinic acetylcholine receptor comprising at least one alpha receptor subunit and at least one beta subunit, wherein said alpha receptor subunit is alpha5, and said beta subunit(s) is selected from the group consisting of beta2, beta3 and beta4 and

measuring the response of said receptor to said drug substance.

16. A method for screening drug substances to determine those drug substances which function as antagonists for nicotinic acetylcholine receptors, said method comprising:

contacting a receptor comprised of a substantially pure neuronal nicotinic acetylcholine receptor comprising at least one alpha receptor subunit and at least one beta subunit, wherein said alpha receptor subunit is alpha5 and said beta subunit(s) is selected from the group consisting of beta2, beta3 and beta4, with:

a fixed concentration of an agonist for a neuronal nicotinic acetylcholine receptor, and increasing concentrations of said drug substance, and

measuring the change in response of said receptor in the presence of said drug substance.

20. A method to identify drug substances which are capable of binding to nicotinic acetylcholine receptors, said method comprising

carrying out a competitive binding assay wherein said drug substance is contacted with neuronal nicotinic acetylcholine receptor subunit alpha5 in the presence of at least one agonist for neuronal nicotinic acetylcholine receptors, and

determining whether or not said drug substance affects the ability of said agonist to bind to said receptor subunit.

☐ 19. Document ID: US 6100046 A

L25: Entry 19 of 33 File: USPT Aug 8, 2000

DOCUMENT-IDENTIFIER: US 6100046 A
** See image for Certificate of Correction **
TITLE: Methods of identifying modulators of alpha9, a novel acetylcholine-gated ion channel receptor subunit

CLAIMS:

1. A method for identifying compounds that bind to alpha9 acetylcholine-gated ion receptor subunit(s), said method comprising:

a) contacting control cells that do not express said alpha9 subunit with a test compound;

b) contacting test cells with said test compound, wherein said test cells are transformed with and express a nucleic acid encoding said alpha9 subunit; and

c) identifying test compounds that bind to said alpha9 subunit by comparing the amount of said test compound that binds to said test cells to the amount of said test compound that binds to said control cells.

7. A bioassay for identifying compounds that are agonists of acetylcholine-gated ion ion receptors comprising at least one alpha9 subunit, said method comprising:

a) contacting cells transformed with, and expressing, a nucleic acid encoding said alpha9 subunit with a test compound, wherein the ability of said test compound to affect the ion channel activity of said receptor is unknown; and thereafter

b) monitoring said cells for changes in ion channel activity, wherein said test compound is determined to be an agonist if the ion channel activity of said receptor is increased in the presence of said test compound.

13. A bioassay for identifying compounds that are antagonists of acetylcholine-gated gated ion receptors comprising at least one alpha9 subunit, said method comprising:

a) contacting cells transformed with, and expressing, a nucleic acid encoding said alpha9 subunit with a known agonist of said alpha9 subunit and a test compound, wherein the ability of said test compound to affect the ion channel activity of said receptor is unknown; and thereafter

b) monitoring said cells for changes in ion channel activity, wherein said test compound is determined to be an antagonist if the ion channel activity of said receptor, in the presence of said agonist, is reduced in the further presence of said test compound.

☐ 20. Document ID: US 6084075 A

L25: Entry 20 of 33 File: USPT Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6084075 A
**** See image for Certificate of Correction ****
TITLE: Agonist and antagonist antibodies to the chemokine receptor-2 (CCR2)

CLAIMS:

7. The antibody according to claim 1, further comprising:

a label to detecting the antibody, the label being selected from the group consisting of radioactive labels, fluorescence labels, enzymatic labels, and affinity tags.

15. The antibody according to claim 8, further comprising:

a label to detecting the antibody, the label being selected from the group consisting of radioactive labels, fluorescence labels, enzymatic labels, and affinity tags.

21. An antibody, comprising:

an antagonist antibody that binds to the extracellular sequence comprising the third third extracellular domain of the monocyte chemoattractant protein-1 chemokine receptor CCR2, thereby blocking responses associated with binding of the CCR-2 chemokine to the extracellular sequence of the receptor.

☐ 21. Document ID: US 6070126 A

L25: Entry 21 of 33 File: USPT May 30, 2000

DOCUMENT-IDENTIFIER: US 6070126 A
TITLE: Immunobiologically-active linear peptides and method of identification

CLAIMS:

1. A method for determining an optimal length of contiguous amino acid residues of a a Ho-Hi-Ho model epitope within a polypeptide, said Ho-Hi-Ho model epitope characterized by a hydrophobic-hydrophilic-hydrophobic motif, the method comprising the steps of:

a) assigning a window average hydropathy value to each amino acid of the polypeptide;

b) generating a hydropathy plot using said window average hydropathy value of each amino acid;

c) fitting each curve segment of said hydropathy plot to a negative cosine function, wherein a specific period number value of said negative cosine function increases within a predetermined chosen period number range after each sequential lagging through said hydropathy plot thereby providing fit-correlation values for each region of amino acid sequence number ranges of said polypeptide when using said specific period number value;

d) generating a potential Ho-Hi-Ho model epitope set for each specific period number value within said chosen period number range, wherein each potential Ho-Hi-Ho model epitope set contains potential Ho-Hi-Ho model epitopes with said amino acid sequence number ranges that have a positive-fit correlation value;

e) ranking each potential Ho-Hi-Ho model epitope with amino acid sequence number range in said potential Ho-Hi-Ho model epitope set according to positive fit-correlation values wherein said the potential Ho-Hi-Ho model epitope with amino acid sequence number range having highest said positive-fit correlation value is ranked number one thereby providing ranked Ho-Hi-Ho model theoretical epitopes for each specific period number value;

f) providing peptides that together span the length of said polypeptide, the peptides having a length from about 15 to about 25 mers;

g) generating experimental data on immunobiologic reactivity of said

peptides;

h) ranking experiment peptides according to experimental immunobiologic reactivity thereby providing a peptide experimental ranking value for each peptide;

i) comparing amino acid residue sequences of said experimental peptide with amino acid residue sequences of said ranked Ho-Hi-Ho model theoretical epitopes wherein a positive correlation of amino acid residue sequences provides the basis for assigning said experimental peptide a theoretical ranking dependent upon the ranking of a corresponding Ho-Hi-Ho model theoretical epitope thereby providing a peptide theoretical ranking for each peptide when using said potential Ho-Hi-Ho model epitope set derived from a specific period number value;

j) calculating a correlation coefficient by correlating said peptide experimental ranking to said peptide theoretical ranking for each peptide when using a potential Ho-Hi-Ho model epitope set derived from a specific period number value;

k) determining a statistical p-value of said correlation coefficient;

l) determining said specific period number value having lowest statistical p-value; and

m) determining an optimal length of a Ho-Hi-Ho model epitope by assigning said specific period number value with said lowest statistical p-value to said Ho-Hi-Ho model epitope.

5. The method according to claim 1 wherein said experimental data on immunogenic reactivity of said peptides may be generated by a testing method selected from the group consisting ELISA, RIA, surface plasmon resonance, and immunofluorescence.

7. The method according to claim 1 wherein said polypeptide contains at least one Ho-Hi-Ho model epitope selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN-.alpha., IFN-.beta., IFN-65 , CD2, CD3, CD4, CD5, CD8, CD11a, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54, CD56, K2, K1, P.beta., O.alpha., M.alpha., M.beta.2, M.beta.1, LMP1, TAP2, LMP7, TAP1, O.beta., IA.beta., IA.alpha., IE.beta., IE.beta.2, IE.alpha., CYP21, C4B, CYP21P, C4A, Bf, C2, HSP, G7a/b, TNF-.alpha., TNF-.beta., D, L, Qa, Tla, COL11A2, DP.beta.2, DP.alpha.2, DP.beta.1, DP.alpha.1, DN.alpha., DM.alpha., DM.beta., LMP2, TAPi1, LMP7, DO.beta., DQ.beta.2, DQ.alpha.2, DQ.beta.3, DQ.beta.1, DQ.alpha.1, DR.beta., DR.alpha., HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-F, nerve growth factor, somatotropin, somatomedins, parathormone, FSH, LH, EGF, TSH THS-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF-.beta., GM-CSF, M-CSF, G-CSF1, erythropoietin, .beta.-HCG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Her2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMel 17, tyrosinase related proteins 1 and 2, p97, p53, RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC and MCC, ras, myc, neu, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl and abil,

C1q, C1r, C1s, C4, C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, C1Inh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF, MIRL, CR1, CR2, CR3, CR4, C3a/C4a receptor, C5a receptor, HIV (gag, pol, qp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, .alpha.-TIF, ICP4, ICP8, 1CP35, LAT-related proteins, gB, gC, gD, gE, gH, gI, gJ), influenza (hemagluttinin, neuraminidase, PB1, PB2, PA, NP, M.sub.1, M.sub.2, NS.sub.1, NS.sub.2), papillomaviruses (E1, E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2) adenovirus (E1A, E1B, E2, E3, E4, E5, L1, L2, L3, L4, L5), Epstein-Barr Virus (EBNA), Hepatitis B Virus (gp27.sup.S, gp36.sup.S, gp42.sup.S, p22.sup.c, pol, x)and Nuclear Matrix Proteins.

☐ 22. Document ID: US 5981193 A

L25: Entry 22 of 33 File: USPT Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981193 A
TITLE: Human neuronal nicotinic acetylcholine receptor compositions and methods employing same

CLAIMS:

2. An isolated and purified human neuronal nicotinic acetylcholine receptor subunit encoded by the alpha3-encoding nucleic acid in a plasmid having all of the identifying characteristics of HnAChR.alpha.3 deposited under ATCC Accession No. 68278.

3. An isolated and purified human neuronal nicotinic acetylcholine receptor subunit encoded by the beta2-encoding nucleic acid in a plasmid having all of the identifying characteristics of HnAChR.alpha.3 deposited under ATCC Accession No. 68279.

4. A method of making cells having neuronal nicotinic acetylcholine receptor activity, comprising:

(a) introducing one or more nucleic acid molecules that encode(s) at least one alpha subunit of a neuronal nicotinic acetylcholine receptor and at least one beta subunit of a neuronal nicotinic acetylcholine receptor into eukaryotic cells, wherein the nucleic acid encoding an alpha subunit comprises a sequence of amino acids encoded by the alpha3-encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR.alpha.3 deposited under ATCC ATCC Accession No. 68278, and the nucleic acid encoding the beta subunit comprises a sequence of amino acids encoded by SEQ ID No. 9;

(b) selecting cells from (a) that express the alpha or the beta encoding nucleic acid or express the alpha and beta subunit-encoding nucleic acid; and

(c) detecting neuronal nicotinic acetylcholine receptor activity in the selected cells, wherein the activity is mediated by a receptor containing one or more of the alpha and beta subunits encoded by said introduced nucleic acid molecules.

5. An isolated nucleic acid molecule, comprising the alpha2-encoding nucleic acid open reading frame that is isolated from a plasmid having all of the identifying characteristics of HnAChR.alpha.2 deposited under ATCC Accession No. 68277.

6. An isolated nucleic acid molecule, comprising a sequence of nucleotides encoding an alpha3 subunit of a human nicotinic acetylcholine receptor that is encoded by the alpha3-encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR.alpha.3 deposited under ATCC Accession No. 68278.

10. A method for screening compounds for activity as nicotinic acetylcholine receptor agonists or antagonists, said method comprising:

contacting a cell of claim 7 with a test compound, and thereafter

monitoring nicotinic acetylcholine receptor activity of the cell by monitoring the performance of the cell by measuring a performance parameter selected from the group group consisting of the flux of ions through the membrane of the cell, nicotine binding to nicotinic acetylcholine receptors of the cell, or the electrophysiological response of the cells, wherein

the cell expresses a nicotinic acetylcholine receptor that contains a subunit encoded by the nucleic acid molecule.

13. The cell of claim 7, further comprising a reporter gene expression construct; and

the reporter gene expression construct comprises:

a transcriptional control element, and

a reporter gene encoding a transcriptional and/or translational product;

the transcriptional control element, in said cell, is responsive to an intracellular condition that occurs when a human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with

respect to said receptor;

said product can be, directly or indirectly, detected; and

the reporter gene is in operative association with said transcriptional control element.

14. A method for screening test compounds for activity as nicotinic acetylcholine receptor agonists or antagonists, comprising:

comparing the difference in the amount of transcription of a reporter gene in the cells of claim 13 in the presence of the compound with the amount of transcription in the absence of the compound or with the amount of transcription in the control cells that do not express nicotinic acetylcholine receptors, but contain the reporter gene expression construct, wherein compounds that exhibit activity as agonists or antagonists are identified.

20. An isolated nucleic acid molecule, comprising a sequence of nucleotides encoding a beta2 subunit of a human nicotinic acetylcholine receptor that is encoded by the beta2-encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR.beta.2 deposited under ATCC Accession No. 68279 or the sequence of nucleotides set forth as nucleotides 1-1521 in SEQ ID No. 9.

28. An isolated and purified protein encoded by the alpha2-encoding nucleic acid open reading frame that is isolated from a plasmid having all of the identifying characteristics of HnAChR.alpha.2 deposited under ATCC Accession Number 68277.

29. An isolated and purified subunit of a human nicotinic acetylcholine receptor encoded by the alpha3-encoding nucleic acid open reading frame that is isolated from a plasmid having all of the identifying characteristics of HnAChR.alpha.3 deposited under ATCC Accession Number 68278.

30. An isolated and purified subunit of a human nicotinic acetylcholine receptor encoded by the beta2-encoding nucleic acid open reading frame that is isolated from a plasmid having all of the identifying characteristics of HnAChR.beta.2 deposited under ATCC Accession Number 68279 or the sequence of nucleotides set forth as nucleotides 1-1521 of SEQ ID NO:9.

31. A plasmid having all of the identifying characteristics of the plasmid deposited deposited under ATCC Accession No. 68277.

40. A plasmid having all of the identifying characteristics of the plasmid deposited deposited under ATCC Accession No. 68278.

41. A plasmid having all of the identifying characteristics of the plasmid deposited deposited under ATCC Accession No. 68279.

☐ 23. Document ID: US 5935781 A

L25: Entry 23 of 33 File: USPT Aug 10, 1999

DOCUMENT-IDENTIFIER: US 5935781 A
TITLE: Apolipoprotein E polymorphism and treatment of Alzheimer's disease

CLAIMS:

1. A method for the identification of human subjects with cognitive impairments to be responsive to a cholinomimetic drug comprising determining the number of copies of apoE4 gene alleles in said subject and wherein the absence of at least one apoE4 gene allele indicates a predisposition to respond to a cholinomimetic drug.

2. A method of treating human subjects with cognitive impairments comprising identifying a subject according to the method of claim 1 and administering a therapeutically effective amount of a cholinomimetic drug wherein administration of the cholinomimetic drug improves cognitive performance.

3. The method of claim 2 wherein said cholinomimetic drug is selected from the group consisting of inhibitors of acetylcholine degradation, inducers of acetylcholine synthesis, acetylcholine agonists or mimics, and muscarinic M2-receptor antagonists.

4. The method of claim 1 wherein the number of copies of apoE4 gene alleles is determined indirectly by determining the presence of apoE2 and/or apoE3 gene alleles alleles using appropriate apoE2 and apoE3 probes.

☐ 24. Document ID: US 5879896 A

L25: Entry 24 of 33 File: USPT Mar 9, 1999

DOCUMENT-IDENTIFIER: US 5879896 A
TITLE: Method of screening for inhibitors of human thyrotropin releasing hormone (TRH) receptor

CLAIMS:

1. A method of screening for a compound that inhibits binding of TRH to a human TRH receptor, or a salt thereof, comprising contacting a TRH receptor protein obtained from a cell transformed with an expression vector containing a DNA encoding a TRH receptor having the amino acid sequence of SEQ ID NO: 1, or a sufficient portion thereof to bind TRH, or the salt thereof, with the compound to be screened and TRH, and comparing binding between TRH and the TRH receptor in the absence and presence of the compound, wherein less binding between the TRH and the receptor in the presence of the compound than in the absence of the compound is indicative of the compound inhibiting binding between TRH and the receptor.

3. A method of screening for a compound that inhibits binding of TRH to a human TRH receptor, or a salt thereof, comprising comparing the amount of a labeled ligand bound to a receptor protein free of human tissue and having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof by steps (a) and (b);

(a) contacting the labeled ligand with the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or a salt thereof,

(b) contacting the labeled ligand and a test compound with the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof, wherein a decrease in labeled ligand binding in step (b) indicates that the compound inhibits binding of TRH to a human TRH receptor, or a salt thereof, further wherein said receptor is from a cell transformed with an expression vector containing a DNA encoding the amino acid sequences of SEQ ID NO:1, NO:1, or a sufficient portion thereof to bind TRH.

6. A method of screening for a compound that inhibits binding of TRH to a human TRH receptor, or a salt thereof, comprising comparing the amounts of a labeled ligand bound to a receptor protein free of human tissue having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof in steps (a) and (b);

(a) contacting the labeled ligand with the TRH receptor protein which is expressed on a cell membrane, said cell membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH,

(b) contacting the labeled ligand and a test compound with the TRH receptor protein which is expressed on a cell membrane, said cell membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, wherein a decrease in labeled ligand binding in step (b) indicating that the compound inhibits binding of TRH to a human TRH receptor, or a salt thereof.

8. A method of screening for a TRH receptor agonist or antagonist comprising measuring cell stimulating activities through a TRH receptor determined from the following steps (a) and (b);

(a) contacting a test compound with a cell transformed with a DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not comprise the receptor, wherein said compound having cell stimulating activity in the test screen but not the control indicates that said compound is a TRH receptor agonist,

(b) contacting a TRH receptor-activating compound and a test compound with a cell transformed with a DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not comprise the receptor, wherein a decrease in cell stimulating activity by the TRH receptor-activating compound in the test screen but not the control indicates that the compound is a TRH receptor antagonist, and further wherein the cell stimulating activities are selected from the group consisting of mobilization of calcium in the cells, hyper metabolism of inositol phosphate, arachidonic acid releasing activity, acetylcholine releasing activity, activation of adenylate cyclase and activation of c-fos.

9. A method of screening for a TRH receptor agonist or antagonist comprising measuring cell stimulating activities through a TRH receptor in steps of (a) and (b);

(a) contacting a test compound with the TRH receptor protein which is expressed on a cell membrane, said membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not express the DNA, wherein said compound having cell stimulating activity in the test screen but not the control indicates that said compound is a TRH receptor agonist,

(b) contacting a TRH receptor-activating compound and a test compound with the TRH receptor protein which is expressed on a cell membrane, said membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not comprise the receptor, wherein a decrease in cell stimulating activity by the TRH receptor-activating compound in the test screen of step (b) but not the control indicates that the compound is a TRH TRH receptor antagonist, and further wherein the cell stimulating activities are selected from the group consisting of mobilization of calcium in the cells, hyper metabolism of inositol phosphate, arachidonic acid releasing activity, acetylcholine, activation of adenylate cyclase and activation of c-fos.

13. A method of screening for a compound that inhibits binding of TRH to a TRH receptor, or a salt thereof, comprising contacting a TRH receptor protein encoded by the nucleotide sequence of SEQ ID NO:2 with the compound to be screened and TRH and determining whether the compound inhibits the binding of TRH to the TRH receptor, wherein said receptor is from a cell transformed with an expression vector containing the nucleotide sequence of SEQ ID NO:2.

14. A method of screening for a compound that inhibits the binding of TRH to a human human TRH receptor, or a salt thereof, comprising comparing the amount of a labeled ligand bound to the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof determined from the following steps (a) and (b);

(a) contacting the labeled ligand with a cell which contains the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof,

(b) contacting the labeled ligand and a test compound with a cell which contains the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof, wherein the cell is transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof which bind TRH, and the cell is selected from the group consisting of monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L-cell, mouse myeloma cell and human FL cell, and wherein a decrease in labeled ligand binding compared with step (a) indicates that the compound inhibits binding of TRH to a human TRH receptor, or a salt thereof.

15. A method of screening for a compound that inhibits the binding of TRH to a human human TRH receptor, or a salt thereof, comprising comparing the amount of a labeled ligand bound to the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof by steps (a) and (b);

(a) contacting said labeled ligand with a membrane fraction of a cell transformed with a DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH,

(b) contacting said ligand and said test compound with the membrane fraction of the cell transformed with the DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, wherein the cell is selected from the group consisting of monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L-cell, mouse myeloma cell and human FL cell, and wherein a decrease in labeled ligand binding in step (b) indicates that the compound inhibits binding of TRH to a human TRH receptor, or a salt thereof.

16. A method of screening for a compound that inhibits binding of TRH to a human TRH TRH receptor, or a salt thereof, comprising comparing the amounts of a labeled

ligand bound to a receptor protein having the amino acid sequence of SEQ ID NO:1, or or a sufficient portion thereof to bind TRH, or the salt thereof in steps (a) and (b);

(a) contacting the labeled ligand with the TRH receptor protein which is expressed on a cell membrane, said cell membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, (b) contacting the labeled ligand and a test compound with the TRH receptor protein which is expressed on a cell membrane, said cell membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, wherein the cell is selected from the group consisting of monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L-cell, mouse myeloma cell and human FL cell, and wherein a decrease in labeled ligand ligand binding in step (b) indicates that the compound inhibits binding of TRH to a human TRH receptor, or a salt thereof.

17. A method of screening for a compound that is a TRH receptor agonist or antagonist comprising measuring cell stimulating activities through a TRH receptor determined from the following steps (a) and (b);

(a) contacting a test compound with a cell transformed with a DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not comprise the receptor, wherein said compound having cell stimulating activity in the test screen but not the control indicates that said compound is a TRH receptor agonist,

(b) contacting a TRH receptor-activating compound and the test compound with a cell transformed with a DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not comprise the receptor, wherein a decrease in cell stimulating activity by the TRH receptor-activating compound in the test screen but not the control indicates that the compound is a TRH receptor antagonist, and further wherein the cell is selected from the group consisting of monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L-cell, mouse myeloma cell and human FL cell.

18. A method of screening for a compound that is a TRH receptor agonist or antagonist comprising measuring cell stimulating activities through a TRH receptor determined from the following steps (a) and (b);

(a) contacting a test compound with a TRH receptor protein which is expressed on a cell membrane, said cell membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell membrane does not comprise the receptor, wherein said compound having cell stimulating activity in the test screen but not the control indicates that said compound is a TRH receptor agonist,

(b) contacting a TRH receptor-activating compound and the test compound with the TRH receptor protein which is expressed on a cell membrane, said cell membrane obtained from a cell transformed with a DNA encoding the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell membrane does not comprise the receptor, wherein a decrease in cell stimulating activity by the TRH receptor-activating compound in the test screen but not the control indicates that the compound is a TRH receptor antagonist, and further wherein the cell is selected from the group consisting of monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L-cell, mouse myeloma cell and human FL cell.

19. A method of screening for a compound that is evaluated as TRH receptor agonist or antagonist that inhibits binding of TRH to a human TRH receptor, or a salt thereof, comprising:

(i) identifying a compound that inhibits binding of TRH to a human TRH receptor, or a salt thereof comprising comparing the amounts of a labeled ligand bound to a receptor protein free of human tissue and having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof by steps (a) and (b);

(a) contacting the labeled ligand with the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, or the salt thereof,

(b) contacting the labeled ligand and a test compound with the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient protein thereof to bind TRH, or the salt thereof, wherein a decrease in labeled ligand binding in step (b) indicates that the compound inhibits binding or TRH to a human TRH receptor, or a salt thereof, and

(ii) determining whether said compound which inhibits binding of TRH to a human TRH receptor, or a salt thereof, is a TRH receptor agonist or TRH receptor antagonist by measuring cell stimulating activities through a TRH receptor comprising contacting said compound with a cell transformed with a DNA encoding the receptor protein having the amino acid sequence of SEQ ID NO:1, or a sufficient portion thereof to bind TRH, referred to as a test screen, and comparing the results to a control wherein the cell does not comprise the receptor, wherein said compound having cell stimulating activity in the test screen but not the control indicates that said compound is a TRH receptor agonist, and said compound having no cell stimulating activity in the test screen and the control indicates that said compound compound is a TRH receptor antagonist, wherein the cell stimulating activities are selected from the group consisting of mobilization of calcium in the cells, hyper metabolism of inositol phosphate, arachidonic acid releasing activity, acetylcholine releasing activity, activation of adenylate cyclase and activation of c-fos.

☐ 25. Document ID: US 5843024 A

L25: Entry 25 of 33 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843024 A
** See image for Certificate of Correction **
TITLE: Solution and process for resuscitation and preparation of ischemically damaged tissue

CLAIMS:

4. The process according to claim 1, wherein the perfusion is performed using a device comprising a laminar or pulsatile pumping system to deliver the buffered physiological solution, and said device further includes a means for providing and controlling perfusion and perfusion pressure; a means for temperature control; a means for providing and controlling introduction of, and venting of, respiratory gases; and a means for testing or collecting for testing the buffered physiological

solution which has already perfused through the organ to monitor and measure a functional characteristic selected from the group consisting of pH, various pressures, flow rate, vascular resistance, chemical constituents, oxygenation, carbon dioxide concentration, oxygen consumption, and a combination thereof.

5. The process according to claim 4, wherein the perfusion is performed using a second device, in conjunction with the first device, to test or collect for testing an organ product diverted from the organ, wherein subsequent measurement of parameters of the organ product relate to organ integrity and function.

9. The process according to claim 6, wherein the perfusion is performed using a device comprising a laminar or pulsatile pumping system to deliver the buffered physiological solution, and said device further includes a means for providing and controlling perfusion and perfusion pressure; a means for temperature control; a means for providing and controlling introduction of, and venting of, respiratory gases; and a means for testing or collecting for testing the buffered physiological solution which has already perfused through the organ to monitor and measure a functional characteristic selected from the group consisting of pH, various pressures, flow rate, vascular resistance, chemical constituents, oxygenation, carbon dioxide concentration, oxygen consumption, and a combination thereof.

10. The process according to claim 9, wherein the perfusion is performed using a second device, operatively associated with the first device, to test or collect for testing an organ product diverted from the organ, wherein subsequent measurement of parameters of the organ product relate to organ integrity and function.

13. The resuscitation solution according to claim 12, wherein the resuscitation solution comprises substrates for endothelial cell-mediated vasodilation, substrates for microvessel vasodilation, and calcium channel blockers, wherein the substrates for endothelial cell-mediated vasodilation comprise acetylcholine and arginine, wherein the substrates for microvessel vasodilation comprise prostacyclin and Mg.sup.+, and wherein the calcium channel blockers comprise adenosine and verapamil.

25. The process according to claim 24, wherein the resuscitation solution comprises substrates for endothelial cell-mediated vasodilation, substrates for microvessel vasodilation, and calcium channel blockers, wherein the substrates for endothelial cell-mediated vasodilation comprise acetylcholine and arginine, wherein the substrates for microvessel vasodilation comprise prostacyclin and Mg.sup.+, and wherein the calcium channel blockers comprise adenosine and verapamil.

☐ 26. Document ID: US 5591590 A

L25: Entry 26 of 33 File: USPT Jan 7, 1997

DOCUMENT-IDENTIFIER: US 5591590 A
TITLE: Neuronal nicotinic acetylcholine receptor assay

CLAIMS:

1. A method for screening substances to determine those substances which function as

as agonists for neuronal nicotinic acetylcholine receptors, said method comprising:

contacting a receptor with a substance wherein said receptor comprises a neuronal nicotinic acetylcholine receptor comprising at least one alpha receptor subunit and at least one beta subunit, wherein said alpha receptor subunit(s) is expressed from recombinant DNA, and is selected from the group consisting of alpha2, alpha3, alpha4.1, alpha4.2, and alpha5, and said beta subunit(s) is expressed from recombinant DNA, and is selected from the group consisting of beta2, beta3 and beta4, and

measuring the response of said receptor to said substance.

5. A method for screening substances to determine those substances which function as as antagonists for neuronal nicotinic acetylcholine receptors, said method comprising:

contacting a receptor with a fixed concentration of an agonist for the receptor, and increasing concentrations of a substance, wherein said receptor comprises a neuronal nicotinic acetylcholine receptor comprising at least one alpha receptor subunit and at least one beta receptor subunit, wherein said alpha receptor subunit (s) is expressed from recombinant DNA, and is selected from the group consisting of alpha2, alpha3, alpha4.1, alpha4.2, and alpha5, and said beta receptor subunit(s) is expressed from recombinant DNA, and is selected from the group consisting of beta2, beta3 and beta4; and

measuring the change in response of said receptor in the presence of said substance. substance.

9. A method to identify substances which are capable of binding to neuronal nicotinic acetylcholine receptors, said method comprising

carrying out a competitive binding assay wherein said substance is contacted with at at least one subunit, wherein said subunit is expressed from recombinant DNA, and is selected from the group consisting of neuronal nicotinic acetylcholine receptor subunits alpha2, alpha3, alpha4.1, alpha4.2, and alpha5 in the presence of at least one agonist for neuronal nicotinic acetylcholine receptors, and

determining whether or not said substance affects the ability of said agonist to bind to said receptor subunit.

☐ 27. Document ID: US 5449606 A

L25: Entry 27 of 33 File: USPT Sep 12, 1995

DOCUMENT-IDENTIFIER: US 5449606 A
TITLE: DNA encoding neuronal nicotinic acetylcholine receptor compositions containing the beta 4 subunit

CLAIMS:

11. A method for detecting compounds that bind to nicotinic acetylcholine receptors, receptors, said method comprising contacting test cells with labeled compound, and thereafter determining the presence of labeled compound bound to test cells,

wherein test cells express nicotinic acetylcholine cell surface receptors containing at least one beta4 subunit encoded by the mRNA of claim 10, wherein said mRNA is derived from a source other than said test cell.

12. A method of identifying compounds that are neuronal nicotinic acetylcholine receptor agonists, said method comprising comparing the response of test cells in the presence of said compound, relative to the response of said test cells in the absence of said compound,

wherein test cells express nicotinic acetylcholine cell surface receptors containing at least one beta4 subunit encoded by the mRNA of claim 10, wherein said mRNA is derived from a source other than said test cell.

13. A method of identifying compounds that are neuronal nicotinic acetylcholine receptor antagonists, said method comprising:

contacting test cells with increasing concentrations of said compound in the presence of a fixed concentration of a neuronal nicotinic acetylcholine agonist, and

detecting any difference in the response of test cells to agonist in the presence of of said compound, relative to the response of said test cells in the absence of said compound,

wherein test cells express nicotinic acetylcholine cell surface receptors containing at least one beta4 subunit encoded by the mRNA of claim 10, wherein said mRNA is derived from a source other than said test cell.

14. A method for detecting compounds that bind to nicotinic acetylcholine receptors, receptors, said method comprising contacting test cells with labeled compound, and thereafter determining the presence of labeled compound bound to test cells,

wherein test cells express nicotinic acetylcholine cell surface receptors containing at least one beta4 subunit encoded by the DNA of claim 1, wherein said DNA is derived from a source other than said test cell.

15. A method of identifying compounds that are neuronal nicotinic acetylcholine receptor agonists, said method comprising comparing the response of test cells in the presence of said compound, relative to the response of said test cells in the absence of said compound,

wherein test cells express nicotinic acetylcholine cell surface receptors containing at least one beta4 subunit encoded by the DNA of claim 1, wherein said DNA is derived from a source other than said test cell.

16. A method of identifying compounds that are neuronal nicotinic acetylcholine receptor antagonists, said method comprising:

contacting test cells with increasing concentrations of said compound in the presence of a fixed concentration of a neuronal nicotinic acetylcholine agonist, and

detecting any difference in the response of test cells to agonist in the presence of of said compound, relative to the response of said test cells in the absence of said compound,

wherein test cells express nicotinic acetylcholine cell surface receptors containing containing at least one beta4 subunit encoded by the DNA of claim 1, wherein said DNA is derived from a source other than said test cell.

☐ 28. Document ID: US 5401243 A

L25: Entry 28 of 33 File: USPT Mar 28, 1995

DOCUMENT-IDENTIFIER: US 5401243 A
** See image for Certificate of Correction **
TITLE: Controlled administration of chemodenervating pharmaceuticals

CLAIMS:

1. A method of measuring the denervating activity of a chemodenervating preparation comprising

injecting a quantity of said preparation into a muscle or muscle group of an animal;

permitting said preparation to diffuse within said muscle or muscle group thereby establishing a denervation field; and

determining the extent of the denervation field induced by said quantity of the preparation within said muscle or muscle group.

2. The method of claim 1 wherein the step of determining the extent of the denervation field is performed by measuring inhibition of acetylcholine release about the site of injection.

3. The method of claim 2 comprising determining the local concentration of acetylcholinesterase in regions of said muscle spaced apart from said site of injection.

5. The method of claim 1 wherein the step of determining the extent of the denervation field is performed by determining the extent of inhibition of muscle stimulation in regions of said muscle spaced apart from said site of injection by electrophysiologic testing.

6. The method of claim 5 comprising determining the extent of effective muscular stimulation by single fiber electromyography.

7. The method of claim 1 wherein the step of determining the extent of the denervation field is performed by determining the density of acetylcholine receptors receptors or acetylcholinesterase in regions of said muscle spaced apart from said site of injection.

9. The method of claim 1 wherein the step of determining the extent of the dernervation field is performed by measuring the change in muscle fiber diameter.

10. The method of claim 9 wherein the variation in muscle fiber diameter is

determined by obtaining a biopsy of the treated muscle or muscle group at various distances from the site of injection and determining the mean muscle fiber diameter and diameter variation thereof.

11. The method of claim 1 wherein the muscle or muscle group comprises an eye muscle or muscle group, and wherein the step of determining the extent of the dernervation field is performed by observing the degree of ptosis or globe prolapse resulting from said injection.

13. A method of rapidly determining the extent of denervation induced by a unit quantity of a chemodenervating preparation comprising the steps of:

injecting said unit quantity of said chemodenervating preparation into a muscle or muscle group which controls eyelid movement of an animal; and

ascertaining the degree of ptosis or globe prolapse resulting from said injection, wherein said degree of ptosis or globe prolapse is indicative of the spatial extent of denervation induced by said unit quantity.

16. The method of claim 13 wherein said ascertaining step further comprises determining the diffusion capability of said chemodenervating preparation.

☐ 29. Document ID: US 5385915 A

L25: Entry 29 of 33 File: USPT Jan 31, 1995

DOCUMENT-IDENTIFIER: US 5385915 A
** See image for Certificate of Correction **
TITLE: Treatment of amyloidosis associated with Alzheimer disease using modulators of protein phosphorylation

CLAIMS:

6. A method according to claim 2 wherein said indirect modulator of protein kinases is an agonist or antagonist of receptors for intercellular messengers which are known to modulate protein kinases, said receptors selected from the group consisting consisting of adenosine, adrenoreceptors, angiotensin, atrial natriuretic peptide, bombesin, bradykinin, cholecystokinin and gastrin, dopamine, endothelin, GABA, glutamate, histamine, interleukin-1, serotonin, leukotriene, muscarinic acetylcholine, neuropeptide Y, nicotinic acetylcholine, opioid, PAF, prostanoid, purinoceptors, somatostatin, tachykinin, thromkin, vasopressin and oxytocin, and VIP, modulators of and calcium or potassium ion channels.

10. A method according to claim 8 wherein said indirect modulator of protein phosphatase is an agonist or antagonist of receptors for intercellular messengers which are known to modulate protein phosphatases, said receptors selected from the group consisting of adenosine, adrenoreceptors, angiotensin, bombesin, bradykinin, cholecystokinin and gastrin, dopamine, endothelin, GABA, glutamate, histamine,interleukin-1, serotonin, leukotriene, muscarinic acetylcholine, neuropeptide Y, nicotinic acetylcholine, opioid, PAF, prostanoid, purinoceptors, somatostatin, tachykinin, thomkin vasopressin and oxytocin, VIP, and modulators of calcium or potassium ion channels.

16. A method according to claim 12, wherein said indirect modulator of protein kinases is an agonist or antagonist of receptors for intercellular messengers known to modulate protein kinases, said receptors selected from the group consisting of adenosine, adrenoreceptors, angiotensin, atrial natriuretic peptide, bombesin, bradykinin, cholecystokinin and gastrin, dopamine, endothelin, GABA, glutamate, histamine, interleukin-1, serotonin, leukotriene, muscarinic acetylcholine, neuropeptide Y, nicotinic acetylcholine, opioid, PAF, prostanoid, purinoceptors, somatostatin, tachykinin, thrombin, vasopressin and oxytocin, VIP, and modulators of potassium or calcium ion channels.

20. A method according to claim 19 wherein said indirect modulator of protein phosphatases is an agonist or antagonist of receptors for intercellular messengers which are known to modulate protein phosphatases, said receptors selected from the group consisting of adenosine, adrenoreceptors, angiotensin, bombesin, bradykinin, cholecystokinin and gastrin, dopamine, endothelin, GABA, glutamate, histamine, interleukin-1, serotonin, leukotriene, muscarinic acetylcholine, neuropeptide Y, nicotinic acetylcholine, opioid, PAF, prostanoid, purinoceptors, somatostatin, tachykinin, thrombin, vasopressin and oxytocin, VIP, and modulators of calcium or potassium ion channels.

26. A method according to claim 22 wherein said indirect modulator of protein kinases is an agonist or antagonist of receptors for intercellular messengers which are known to modulate protein kinases said receptors selected from the group consisting of adenosine, adrenoreceptors, angiotensin, atrial natriuretic peptide, bombesin, bradykinin, cholecystokinin and gastrin, dopamine, endothelin, GABA, glutamate, histamine, interleukin-1, serotonin, leukotriene, muscarinic acetylcholine, neuropeptide Y, nicotinic acetylcholine, opioid, PAF, prostanoid, purinoceptors, somatostatin, tachykinin, thrombin, vasopressin and oxytocin, VIP, and modulators of calcium or potassium ion channels.

30. A method according to claim 28 wherein said indirect modulator of protein phosphatase is an agonist or antagonist of receptors for intercellular messengers which are known to modulate protein phosphatases, said receptors selected from the group consisting of adenosine, adrenoreceptors, angiotensin, bombesin, bradykinin, cholecystokinin and gastrin, dopamine, endothelin, GABA, glutamate, histamine, interleukin-1, serotonin, leukotriene, muscarinic acetylcholine, neuropeptide Y, nicotinic acetylcholine, opioid, PAF, prostanoid, purinoceptors, somatostatin, tachykinin, thrombin vasopressin and oxytocin, VIP, and modulators of calcium and potassium ion channels.

33. A method of screening for an agent that modulates amyloid formation comprising contacting mammalian cells with an agent suspected of being capable of modulating the phosphorylation of proteins and detecting for alterations in the degradation of APP or changes in .beta./A.sub.4 peptide production comprising the steps of:

(a) providing mammalian cells or tissue sections in culture;

(b) optionally, radioactively labeling proteins produced by the mammalian cells during anabolism; then

(c) allowing the mammalian cells to continue metabolizing in a suitable, label-free media;

(d) contacting the mammalian cells at the start of or during step (c) with an agent suspected of being capable of modulating phosphorylation of proteins that occurs during cell metabolism;

(e) lysing the mammalian cells;

(f) immunoprecipitating the optionally labeled APP fragments moieties with an

antibody against APP; and

(g) comparing the immunoprecipitated APP or APP fragments to standard APP or APP fragments to detect changes in APP degradation and .beta./A.sub.4 peptide production.

34. A method of screening for an agent that modulates amyloid formation comprising administering to a normal or transgenic whole animal an agent suspected of being capable of modulating phosphorylation of proteins and detecting neurodegenerative changes in APP processing, or changes in .beta./A.sub.4 peptide production, in the brain of the animal comprising the steps of:

(a) providing mammalian cells or tissue sections from said animal in culture;

(b) optionally, radioactively labeling proteins produced by the mammalian cells during anabolism; then

(c) allowing the mammalian cells to continue metabolizing in a suitable, label-free media;

(d) contacting the mammalian cells at the start of or during step (c) with an agent suspected of being capable of modulating phosphorylation of proteins that occurs during cell metabolism;

(e) lysing the mammalian cells;

(f) immunoprecipitating the optionally labeled APP fragments moieties with an antibody against APP; and

(g) comparing the immunoprecipitated APP or APP fragments to standard APP or APP fragments to detect changes in APP degradation and .beta./A.sub.4 peptide production.

☐ 30. Document ID: US 5278045 A

L25: Entry 30 of 33 File: USPT Jan 11, 1994

DOCUMENT-IDENTIFIER: US 5278045 A
TITLE: Method and compositions to screen compounds for enhancement of the cholinergic, dopaminergic and serotonergic function

CLAIMS:

1. A screening method to evaluate a compound for enhancing endogenous stimulus-induced neurotransmitter release, said neurotransmitter being selected from the group consisting of acetylcholine, dopamine, or serotonin, the method comprising:

(a) incubating unlabeled compound with radiolabeled 3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one, and a substantially purified protein extracted from brain membranes which enhances the release of said neurotransmitters, the protein having a binding affinity constant of at least 2.9.+-.0.8 nM for tritiated 3,3-bis(4-

pyridinylmethyl)-1-phenylindolin-2-one as determined by a Scatchard analysis and said protein being trypsin sensitive and phospholipase C insensitive; and

(b) determining the extent to which the unlabeled compound inhibits the binding of the radiolabeled compound with the protein.

☐ 31. Document ID: US 5183462 A

L25: Entry 31 of 33 File: USPT Feb 2, 1993

DOCUMENT-IDENTIFIER: US 5183462 A
TITLE: Controlled administration of chemodenervating pharmaceuticals

CLAIMS:

1. A method of standardizing dosages of a chemodenervating preparation by relating extent of denervation in a muscle or muscle group of an experimental animal to a unit quantity of said preparation comprising the steps of injecting a unit quantity of said preparation into said muscle or muscle group of an experimental animal, permitting said preparation to diffuse within said muscle or muscle group about the site of said injection and to physiologically inhibit acetylcholine release, and determining the spatial extent of inhibition of acetylcholine release about the site site of injection within said muscle or group per said unit quantity of said preparation, thereby providing a reliable indicator to a clinician of a volume of said muscle or muscle group which is denervated by a quantity of said preparation.

2. The method of claim 1 comprising determining the local concentration of acetylcholinesterase in regions of said muscle spaced apart from said site of injection.

4. The method of claim 1 comprising determining the extent of inhibition of muscle stimulation in regions of said muscle spaced apart from said site of injection by electrophysiologic testing.

5. The method of claim 1 comprising determining the density of acetylcholine receptors or acetylcholinesterase in regions of said muscle spaced apart from said site of injection.

8. A method of selectively chemically partially denervating a predetermined volume of a muscle or muscle group without inducing significant paresis in muscle tissue adjacent said predetermined volume, the method comprising the steps of:

providing a chemodenervating pharmaceutical characterized in that a unit quantity of said pharmaceutical has been determined to denervate said predetermined volume of a muscle or muscle group;

determining a dose of said pharmaceutical required to denervate the predetermined volume of muscle without inducing significant paresis in muscle tissue adjacent said predetermined volume;

injecting said dose directly into a point within said predetermined volume; and

permitting said dose to diffuse throughout said predetermined volume to induce partial denervation thereof.

12. A method of decreasing spasm or involuntary contraction in a muscle or group of muscles of a patient induced by pathologic neural stimulation caused by cerebral palsy or multiple sclerosis, the method comprising:

providing a chemodenervating pharmaceutical characterized in that a unit quantity of of said pharmaceutical has been determined to denervate a predetermined volume of said muscle or muscle group;

determining a dose of said pharmaceutical required to denervate the predetermined volume of muscle without inducing significant paresis in muscle tissue adjacent said predetermined volume;

injecting directly into a point of said muscle or muscle group of said patient suffering from cerebral palsy or multiple sclerosis said dose of said chemodenervating pharmaceutical to decrease the spasm and involuntary contraction in said muscle or muscle group.

13. A method of decreasing tremor, rigidity or spasticity in a muscle or group of muscles of a patient afflicted with Parkinson's disease, the method comprising:

providing a chemodenervating pharmaceutical characterized in that a unit quantity of said pharmaceutical has been determined to denervate a predetermined volume of said muscle or muscle group;

determining a dose of said pharmaceutical required to denervate the predetermined volume of muscle without inducing significant paresis in muscle tissue adjacent said predetermined volume;

injecting directly into said muscle or muscle group of said patient afflicted with Parkinson's disease said dose of said pharmaceutical to decrease said rigidity or spasticity in said muscle or muscle group.

14. A method of cosmetically decreasing facial wrinkling in a patient induced by contraction of an underlying facial muscle or group of muscles, the method comprising:

providing a chemodenervating pharmaceutical characterized in that a unit quantity of said pharmaceutical has been determined to denervate a predetermined volume of said muscle or muscle group;

determining a dose of said pharmaceutical required to denervate the predetermined volume of muscle without inducing significant paresis in muscle tissue adjacent said predetermined volume;

injecting directly into said facial muscle or muscle group of said patient having said wrinkling said dose of said pharmaceutical to denervate said muscle thereby temporarily reducing the wrinkling caused by contraction of said muscle or muscle group.

☐ 32. Document ID: US 4603382 A

L25: Entry 32 of 33 File: USPT Jul 29, 1986

DOCUMENT-IDENTIFIER: US 4603382 A
TITLE: Dynamic buffer reallocation

CLAIMS:

1. In a data storage system for handling a plurality of independent data transfers wherein each of said data transfers includes transferring blocks of data in a predetermined succession of such blocks of data; a data buffer having a plurality of of allocatable data storage segments, each of said segments having a plurality of addressable data storage registers and for storing one or more of said blocks of data; transfer means coupled to the data buffer for effecting transfer of said blocks of data ino and out of said data buffer in said plurality of independent data data transfers and adapted to be connected to data source-sinks for transferring such blocks of data with any connected data source-sinks, respectively, in said independent data transfers;

the improvement including, in combination:

buffer record table means connected to said data buffer and to said transfer means for storing indications of data stored in the buffer and the available data storage registers for each of said data storage segments;

system means for indicating a predetermined elapsed time;

block length means connected to said data buffer and to said transfer means for separately indicating for each of said data storage segments a length of the data blocks currently being stored in the respective data storage segments;

checking means connected to said block length means, said system means and to said buffer record table means for responding to said system means indicating a predetermined elapsed time for accessing the stored indications of said buffer record table means and the length indications of said block length means for identifying ones of said data storage segments respectively allocated to said independent data transfers, which have stored given blocks of data exceeding a predetermined block length and which relates to a one of said independent data transfers for allocating an additional data storage segment to a one of said one's independent data transfer; and

allocation control means connected to said checking means, to said buffer record table means and to said data buffer and being responsive to said checking means indicating said given blocks to allocate an additional one of data storage segments to a predetermined one of said independent data transfer.

2. The data storage system set forth in claim 1 further including, in combination:

intensity means connected to said transfer means and to said buffer means to sense said intensity of activity individually of said data storage segments and recording said sensed indications of intensity of activity for each of said data storage segments; and

said checking means having reallocate screening means connected to said intensity means for comparing the recorded intensities of said data storage segments and indicating which one of said data storage segments has the greatest recorded

intensity and selection means in the checking means responsive to said greatest intensity indication to select a one of said identified data storage segments and to to identify said one independent data transfer as being the independent data transfer holding allocation of said selected data storage segment.

4. The data storage system set forth in claim 3 further including, in combination:

buffer status table means connected to said buffer for storing indications of the status of each of said data storage segments wherein status shows allocation to respective ones of said independent data transfers, availability for allocation and whether allocated with another one of said data storage segments to a one of said independent data transfers, hereafter called paired dication, and connected to said allocation control means for receiving allocation changes and storing same as status and indicating said stored status;

availability means in said checking means connected to said buffer status table means, to said screening means and to said reset means for responding to said buffer buffer record table means indicating that none of said data storage segments are available for allocation and to inhibit said reset means and said allocation means from allocating any of said data storage segments while said none indication is active; and

deallocation means connected to said buffer and to said availability means for deallocating ones of said data storage segments from said independent data transfers and then activating said availability means to remove said none indication.

6. In the data storage system set forth in claim 5, further including, in combination:

activity measuring means connected to said transfer means for recording an intensity intensity of activity for each of said data storage segments; and

said checking means being connected to said activity measuring means for responding to a maximal one of said recorded intensities of activity to select a one of said independent data transfers related to said identified data storage segments as said predetermined one of said independent data transfers.

10. In a data storage system for handling a plurality of independet data transfers wherein each of said data transfers includes transferring blocks of data in a predetermined succession of such blocks of data; a data buffer having a plurality of allocatable data storage segments, each of said segments having a plurality of registers for storing one or more of said blocks of data; transfer means coupled to the data buffer for effecting transfer of said blocks of data into and out of said data buffer in said plurality of independent data transfers in a plurality of successions of transfers of blocks of data and adapted to be connected to data source-sinks for transferring such blocks of data with any connected data source-sinks;

the improvement including, in combination:

buffer record table means connected to said data buffer and to said transfer means for storing indications of data stored and the available data storage registers for each of said data storage segments;

system means coupled to said transfer means for counting the number of successions of blocks of data being transferred with a first one of said source-sink means and said data buffer by all of said independent data transfers;

value means storing and indicating a predetermined count;

compare means connected to said system means and to said value means for determining determining and indicating when said count equals or exceeds said predetermined count;

CCR generating means connected to said buffer record table means and to said transfer means for indicating a system check point when an ensuing transfer of a block of data with a one of said data storage segments cannot be successfully completed;

CCR counting means connected to said CCR generating means for separately counting each said indicated unsuccessful ensuing transfer of a block of data;

checking means connected to said compare means, said CCR counting means and to said buffer record table means for responding to said compare means indicating said system check point for accessing the stored indications of said buffer record table means and said separate CCR counts for identifying a one of said data storage segments storing given blocks of data which relate to a one of said independent data data transfers having allocation of a data storage segment with a largest one of said CCR counts for allocating an additional data storage segnt to said one independent data transfer;

allocation control means connected to said checking means, to said buffer record table means and to said data buffer to allocate an additional data storage segment to said one independent data transfer; and

reset means coupled to said CCR counting means and to said checking means for resetting said CCR counts each time said checking means responds to said compare means.

12. In the data storage system set forth in claim 11, further including, in combination:

said one source-sink means being a plurality of addressable data storage devices;

said CCR counting means counting the CCR's for each of said plurality of addressable data storage devices as being the CCR counts for said independent data transfers, respectively; and

availability means connected to said data buffer and to said checking means for determining and indicating to said checking means whether any of said data storage segments are available for reallocation or are not allocated and said checking means having inhibit means responsive to said indication for preventing any reallocation if none of the data storage segments are not allocated or not available for allocation.

14. In a data storage system for conducting a plurality of independent data transfers wherein each of said data transfers includes transferring blocks of data in a predetermined succession of such blocks of data; a data buffer having a plurality of allocatable data storage segments, each of said segments having a plurality of registers for storing one or more of said blocks of data; transfers means coupled to the data buffer for effecting transfer of said blocks of data into and out of said data buffer in said plurality of independent data transfers and adapted to be connected to data source-sinks for transferring such blocks of data with any connected data source-sinks;

the improvement, including, in combination:

buffer record table means connected to said data buffer and to said transfer means for storing indications of data stored and available data storage registers for

each of said data storage segments;

system means connected to said transfer means for measuring elapsed time of all data transfers in said transfer means and averaging the measured elapsed times for indicating a normalized predetermined elapsed time;

checking means connected to said system means and to said buffer record table means for responding to said system means indicating a normalized predetermined elapsed time for accessing the stored indications of said buffer record table means having identifying means for identifying a one of said data storage segments storing given blocks of data and which has predetermined available data storage registers and which relate to a one of said independent data transfers for allocating an additional data storage segment to said one independent data transfer; and

allocation control means connected to said checking means, to said buffer record table means and to said data buffer to allocate an additional data storage segment for said one independent data transfer.

15. In the data storage system set forth in claim 14, further including, in combination:

said allocation control means including contention detection means connected to said data buffer for detecting and indicating contention for allocation of one of said data storage segments for more than one of said independent data transfers;

contend means connected to sad contention detection means in said allocation control means and to said checking means for sensing and storing as a contention indication any attempted allocation of any given data storage segment by said allocation control means that fails because said given data storage segment is allocated and for inhibiting said checking means so long as said contention indication is stored; and

means connected to said contend means for resetting said contention indication whereby said checking means is no longer inhibited.

16. In the data storage system set forth in claim 14, further including, in combination:

intensity means connected to said transfer means for measuring and storing intensity indications representing the intensity of data storage activity for respective ones of said data storage elements;

said checking means including comparing means connected to said intensity means for receiving said intensity indications for identifying a one of said data storage elements having a maximum intensity of data storage activities and connected to said identifying means for causing said identifying means to identify said maximum intensity data storage segment as being used in the independent data transfer which is identified as said one independent data transfer.

20. In the data storage system set forth in claim 19, further including, in combination:

said allocation program indicia further including program indicia for enabling said programmable digital processor for allocating ones of said data storage segments which are not additional ones of said data storage segments and for detecting and indicating as a contention any attempted allocation requiring a deallocation before the attempted allocation can be completed;

said checking program indicia further including program indicia for enabling said programmable digital processor for examining said contention indication and or

responding to said contention indication to prevent reallocation until after said contention indication has been removed; and

deallocation program indicia in said control store for enabling said programmable digital processor to deallocate said allocated ones of said data storage segments and having program indicia for enabling said programmable digital processor to remove said contention indication when any one of said allocated data storage segments has been deallocated.

23. In a machine-implemented method of operating a data buffer having a plurality of of allocatable data storage segments;

including the steps of:

allocating predetermined ones of said data storage segments for respective independent data transfers;

transferring data by said independent data transfers in blocks of data;

measuring the length of blocks of data being transferred in said respective independent data transfers;

memorizing the amount of data stored and the free space available for data storage in each of said data storage segments as data storage status;

respectively counting the number of data transfer delays encountered with said independent data transfers as caused by data buffer segment status respectively for data stored in the data buffer and the free data storage space in the respective data storage segments when not meeting the data storage requirements of any given data transfer;

establishing a normalized time period related to the activity of said data buffer for all of said independent data transfers;

measuring elapsed time in a normalized manner;

comparing said measured time with said established time period, when said measured time is less than said established time continue transferring data but when said measured time exceeds or equals said established time, then comparing for each of said data storage segments the number of delays counted and for the data storage segment having the greatest number of said delays counted allocating an additional one of said data storage segment for the independent data transfer having allocation of said data storage segment with said greatest number of delays.

24. In the method set forth in claim 23, further including the steps of:

in said measuring elapsed time step, counting the number of accesses to said data storage segments as an indication of normalized elapsed time;

in said estalishing step, setting a count value as representing said normalized elapsed time period; and

in said comparing step, comparing said set count value with said access count as representing the comparison of said measured time with said established time period.

25. In the method set forth in claim 23, further including the steps of:

attempting to allocate a predetermined one of said data storage segments for a one of said independent data transfers and detecting that none of said data storage

segments are available to be said predetermined one data storage segment and setting setting a contention indication signifying said contention for allocation;

deallocating a one of said allocated data storage segments and removing said contention indication;

delaying said comparing step until after said contention indication has been removed removed so that said deallocation step is completed before any reallocation steps are effected.

☐ 33. Document ID: US 4433058 A

L25: Entry 33 of 33 File: USPT Feb 21, 1984

DOCUMENT-IDENTIFIER: US 4433058 A
TITLE: Membrane receptor assay

CLAIMS:

1. A method for determining in an aqueous assay medium the presence of acetylcholine acetylcholine in a sample suspected of containing said acetylcholine, said method employing a cell surface membrane receptor to said acetylcholine which is bound to said membrane and insoluble in said assay medium and a labeled antagonist to said acetylcholine, wherein said acetylcholine and said labeled antagonist specifically compete for the binding sites of said membrane-bound receptor and said label provides a detectable signal, the measured signal being related to the amount of label bound to receptor and unbound label;

said method comprising:

combining substantially simultaneously in an aqueous buffered medium said membrane-bound receptor, said sample and said labeled antagonist;

incubating for sufficient time to allow for competition between labeled antagonist and acetylcholine resulting in partitioning of said labeled antagonist between said membrane-bound receptor and said assay medium in proportion to the amount of acetylcholine in said medium; and

determining the level of signal as a result of said partitioning.

2. A method according to claim 1, wherein said label is a radionuclide and said determining involves separating labeled antagonist bound to receptor and unbound labeled antagonist.

5. A method according to claim 1, including the additional step after said incubating step of absorbing said aqueous medium with an absorbent, freeing said absorbent of unbound labeled antagonist, and determining the signal from said label bound to said absorbent.

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R2      2987   X       DC=C17.300. (CONNECTIVE TISSUE DISEASES)
R3      3548   R   61  BONE DISEASES, DEVELOPMENTAL
R4        61   N    9  ACNE KELOID
R5      2173   N    3  ALPHA 1-ANTITRYPSIN DEFICIENCY
R6      5917   N    7  ARTHRITIS, JUVENILE RHEUMATOID
R7     54569   N   15  ARTHRITIS, RHEUMATOID
R8       130   N    7  CAPLAN'S SYNDROME
R9      2340   N    9  CARTILAGE DISEASES
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R18       85   N   12  ERYTHEMA INDURATUM
R19      569   N    5  FELTY'S SYNDROME
R20       46   N    5  GANGLION CYSTS
R21      307   N    5  GRANULOMA ANNULARE
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R27    30375   N   11  LUPUS ERYTHEMATOSUS, SYSTEMIC
R28     2274   N    8  LUPUS NEPHRITIS
R29      156   N   17  LUPUS VASCULITIS, CENTRAL NERVOUS SYSTEM
R30     3152   N   13  MARFAN SYNDROME
R31     1153   N    4  MIXED CONNECTIVE TISSUE DISEASE
R32      201   N   13  MUCINOSES
R33      145   N    7  MUCINOSIS, FOLLICULAR
R34     1866   N   14  MUCOPOLYSACCHARIDOSES
R35      715   N   14  MUCOPOLYSACCHARIDOSIS I
R36      494   N   13  MUCOPOLYSACCHARIDOSIS II

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R39      249   N    8  MUCOPOLYSACCHARIDOSIS VI
R40      168   N    7  MUCOPOLYSACCHARIDOSIS VII
R41       76   N    3  MYOFIBROMA
R42     3486   N    5  MYXEDEMA
R43      432   N    5  NECROBIOSIS LIPOIDICA
R44       40   N    5  NECROBIOTIC DISORDERS
R45      478   N   52  NEOPLASMS, CONNECTIVE TISSUE
R46      698   N    9  NOONAN SYNDROME
R47     5842   N   11  OSTEOCHONDRITIS
R48     2699   N   10  OSTEOGENESIS IMPERFECTA

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R49      301   N    4  OSTEOPOIKILOSIS
R50     2031   N    8  PANNICULITIS
?s r1:r50
      S1  144397  R1:R50
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Ref    Items Type  RT  Index-term
R1        92   N    8  PANNICULITIS, LUPUS ERYTHEMATOSUS
R2       794   N    4  PANNICULITIS, NODULAR NONSUPPURATIVE
R3       118   N    5  PANNICULITIS, PERITONEAL
R4       985   N    6  PENILE INDURATION
R5       801   N    4  POLYCHONDRITIS, RELAPSING
R6      1667   N    9  POLYMYALGIA RHEUMATICA
R7       925   N   12  PSEUDOXANTHOMA ELASTICUM
R8     10170   N   26  RHEUMATIC DISEASES
R9       673   N    4  RHEUMATOID NODULE
R10      249   N    4  SCLEREDEMA ADULTORUM
R11       38   N    3  SCLERODERMA, DIFFUSE
R12       10   N    4  SCLERODERMA, LIMITED

          Enter P or PAGE for more
?p

Ref    Items Type  RT  Index-term
R13     2005   N    6  SCLERODERMA, LOCALIZED
R14     9923   N    7  SCLERODERMA, SYSTEMIC
R15     6997   N   11  SJOGREN'S SYNDROME
R16     7880   N   17  SPONDYLITIS, ANKYLOSING
R17      434   N    5  STILL'S DISEASE, ADULT-ONSET
R18      236   N    6  TIETZE'S SYNDROME
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S2      41813   R1:R18
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                   SIGNAL TRANSDUCING)
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E3     92807    17 *SIGNAL TRANSDUCTION
E4     13896       SIGNAL TRANSDUCTION --DRUG EFFECTS --DE
E5         1       SIGNAL TRANSDUCTION --ETHICS --ES
E6      4953       SIGNAL TRANSDUCTION --GENETICS --GE
E7      4365       SIGNAL TRANSDUCTION --IMMUNOLOGY --IM
E8     23777       SIGNAL TRANSDUCTION --PHYSIOLOGY --PH
E9       474       SIGNAL TRANSDUCTION --RADIATION EFFECTS --RE
E10        0     1  SIGNAL TRANSDUCTION, LIGHT
E11        0     1  SIGNAL TRANSDUCTION, MECHANICAL
E12      102       SIGNAL-REGULATED      MAP     KINASES     //EXTRACELLULAR
                   (EXTRACELLULAR SIGNAL-REGULATED MAP KINASES)

          Enter P or PAGE for more
?s e3-e8
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            13896  SIGNAL TRANSDUCTION --DRUG EFFECTS --DE
                1  SIGNAL TRANSDUCTION --ETHICS --ES
             4953  SIGNAL TRANSDUCTION --GENETICS --GE
             4365  SIGNAL TRANSDUCTION --IMMUNOLOGY --IM
            23777  SIGNAL TRANSDUCTION --PHYSIOLOGY --PH
      S3    92807  E3-E8
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Set     Items   Description
S1     144397   R1:R50
S2      41813   R1:R18
S3      92807   E3-E8
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            41813  S2
            92807  S3
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 or enhanc? or increase? or decrease? or change? or alter?)
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           892551  DECREASE?
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           648593  ALTER?
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              287  S6
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?target s7/all

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*File 155: Medline has resumed updating. Please see
HELP NEWS 155 for details.
  File   5:Biosis Previews(R) 1969-2005/Jan W3
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*File   5: Price change effective Jan 1, 2005. Enter HELP
RATES 5 for details.
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RATES 34 for details.
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RATES 73 for details.
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  File 135:NewsRx Weekly Reports 1995-2005/Jan W4
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*File 135: New newsletters are now added. See Help News135 for the
complete list of newsletters.
  File 144:Pascal 1973-2005/Jan W4
         (c) 2005 INIST/CNRS
*File 144: Price change effective Jan 1, 2005. Enter HELP
RATES 144 for details.
  File 149:TGG Health&Wellness DB(SM) 1976-2005/Jan W4
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UD=20041205.
  File 159:Cancerlit 1975-2002/Oct
         (c) format only 2002 Dialog Corporation
*File 159: Cancerlit is no longer updating.
Please see HELP NEWS159.
  File 162:Global Health 1983-2005/Dec
         (c) 2005 CAB International
  File 164:Allied & Complementary Medicine 1984-2005/Feb
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RATES 172 for details.
  File 266:FEDRIP 2004/Oct
         Comp & dist by NTIS, Intl Copyright All Rights Res
  File 369:New Scientist 1994-2005/Jan W3
         (c) 2005 Reed Business Information Ltd.
  File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
*File 370: This file is closed (no updates). Use File 47 for more current
information.
  File 399:CA SEARCH(R) 1967-2005/UD=14206
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Alert feature enhanced for multiple files, etc. See HELP ALERT.
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
(c) 1998 Inst for Sci Info
*File 434: Price change effective Jan 1, 2005. Enter HELP
RATES 434 for details.
File 444:New England Journal of Med. 1985-2005/Jan W4
(c) 2005 Mass. Med. Soc.
File 467:ExtraMED(tm) 2000/Dec
(c) 2001 Informania Ltd.
*File 467: F467 no longer updates; see Help News467. 7.

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S2 3095 'PYK2':'PYK2 EXPRESSION'
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S4 78 E1-E22
S5 3108 (S1 OR S2 OR S3 OR S4)
S6 2597 S5/1999:2005
S7 511 S5 NOT S6
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9/3,KWIC/1 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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129064594 CA: 129(6)64594m PATENT
PYK2 protein tyrosine kinase, screening for drugs for treatment of PYK2 signal transduction-related diseases, and methods for diagnosis of such diseases
INVENTOR(AUTHOR): Lev, Simma; Schlessinger, Joseph
LOCATION: USA
ASSIGNEE: Sugen, Inc.; New York University Medical Center; Lev, Simma; Schlessinger, Joseph
PATENT: PCT International ; WO 9826054 A2 DATE: 19980618
APPLICATION: WO 97US22565 (19971209) *US 32824 (19961211)
PAGES: 86 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-009/12A; C12N-015/12B; C12N-015/63B; C12N-001/21B; C12N-001/19B; C12N-005/10B
DESIGNATED COUNTRIES: AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CU; CZ; DE; DK; EE; ES; FI; GB; GE; GH; HU; ID; IL; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; UA; UG; US; UZ; VN; YU; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; GM; KE; LS; MW; SD ; SZ; UG; ZW; AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA; GN; ML; MR; NE; SN; TD; TG

9/3,KWIC/2 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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128318020 CA: 128(26)318020h PATENT
Cloning of three human RdgB proteins and their diagnostic and therapeutic uses
INVENTOR(AUTHOR): Lev, Sima; Plowman, Gregory D.; Schlessinger, Joseph
LOCATION: USA
ASSIGNEE: Sugen, Inc.; New York University Medical Center; Lev, Sima; Plowman, Gregory D.; Schlessinger, Joseph

PATENT: PCT International ; WO 9816639 A1 DATE: 19980423
APPLICATION: WO 97US17374 (19970926) *US 27337 (19961011)
PAGES: 60 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/12A; C07K-014/435B; C07K-016/18B; C12N-005/12B; A61K-038/17B; C12N-015/63B
DESIGNATED COUNTRIES: AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CU; CZ; DE; DK; EE; ES; FI; GB; GE; GH; HU; ID; IL; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; UA; UG; US; UZ; VN; YU; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; KE; LS; MW; SD; SZ ; UG; ZW; AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA; GN; ML; MR; NE; SN; TD; TG

9/3,KWIC/3 (Item 3 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

125105146 CA: 125(9)105146e PATENT
Protein tyrosine kinase PYK2 cDNA sequence, cloning, and use in diagnosis and gene therapy of signal transduction-related diseases, especially neurological diseases
INVENTOR(AUTHOR): Lev, Sima; Schlessinger, Joseph
LOCATION: USA
ASSIGNEE: Sugen, Inc.; New York University
PATENT: PCT International ; WO 9618738 A2 DATE: 960620
APPLICATION: WO 95US15846 (951206) *US 357642 (941215) *US 460626 (950602)
PAGES: 139 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/54A; C12N-009/12B; C12Q-001/68B; C07K-016/40B; C12N-005/12B; G01N-033/68B; C12Q-001/48B; C07D-041/40B; C07C-255/34B; C07D-215/00B; C07D-239/72B
DESIGNATED COUNTRIES: AM; AT; AU; BB; BG; BR; BY; CA; CH; CN; CZ; DE; DK; EE; ES; FI; GB; GE; HU; IS; JP; KE; KG; KP; KR; KZ; LK; LR; LT; LU; LV; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; TJ; TM
DESIGNATED REGIONAL: KE; LS; MW; SD; SZ; UG; AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA; GN; ML; MR; NE; SN; TD; TG
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12942785 PMID: 8606775

A mechanism for regulation of the adhesion-associated proteintyrosine kinase pp125FAK.

Richardson A; Parsons T

Department of Microbiology, Health Science Center, University of Virginia, Charlottesville 22908, USA.

Nature (ENGLAND) Apr 11 1996, 380 (6574) p538-40, ISSN 0028-0836 Journal Code: 0410462

Erratum in Nature 1996 Jun 27;381(6585) 810

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Focal adhesion kinase (pp125FAK) is a member of a growing family of structurally distinct protein tyrosine kinases that includes the recently identified FakB and **PYK2** /CAKbeta/RAFTK. Activation of pp125FAK has been functionally linked to the formation of focal adhesions, integrin-mediated sites of contact between the cell and the extracellular matrix. The carboxy-terminal domain of pp125FAK is also expressed as a separate protein called pp41/43FRNK (where FRNK represents pp125FAK-related non-kinase). Here we show that pp41/43FRNK acts as an inhibitor of pp125FAK by transiently blocking the formation of focal adhesions on fibronectin and constitutively reducing tyrosine phosphorylation of both pp125FAK and two focal adhesion proteins, tensin and paxillin. These inhibitory effects of pp41/43FRNK are reversed by co-expression of pp125FAK, suggesting that pp125FAK and pp41/43 FRNK compete for a common binding protein(s) whose association with pp125FAK is necessary for signalling by pp125FAK. We propose that pp41/43FRNK functions as an endogenous regulator of pp125FAK, thus providing an unusual means to regulate both tyrosine kinase activity and cellular adhesion to the extracellular matrix.

Descriptors: *Cell Adhesion Molecules--metabolism--ME; *Protein-Tyrosine Kinase--metabolism--ME; Animals; Binding, Competitive; Cell Adhesion; Cell Movement; Cells, Cultured; Chickens; Cytoskeletal Proteins--metabolism--ME; Enzyme Activation; Fibronectins--metabolism--ME; Microfilament Proteins --metabolism--ME; Phosphoproteins--metabolism--ME; Phosphorylation; Protein-Tyrosine Kinase--antagonists and inhibitors--AI; Transfection; Tyrosine--metabolism--ME

CAS Registry No.: 0 (Cell Adhesion Molecules); 0 (Cytoskeletal Proteins); 0 (Fibronectins); 0 (Microfilament Proteins); 0 (Phosphoproteins); 0 (paxillin); 0 (tensin); 55520-40-6 (Tyrosine)

Enzyme No.: EC 2.7.1.- (endogenous substrate pp120); EC 2.7.1.112 (Protein-Tyrosine Kinase)

Record Date Created: 19960520

Record Date Completed: 19960520

13431262 PMID: 9104812

Tyrosine phosphorylation of Pyk2 is selectively regulated by Fyn during TCR signaling.

Qian D; Lev S; van Oers N S; Dikic I; Schlessinger J; Weiss A

Department of Medicine, University of California, San Francisco 94143, USA.

Journal of experimental medicine (UNITED STATES) Apr 7 1997, 185 (7) p1253-9, ISSN 0022-1007 Journal Code: 2985109R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The Src family protein tyrosine kinases (PTKs), Lck and Fyn, are coexpressed in T cells and perform crucial functions involved in the initiation of T cell antigen receptor (TCR) signal transduction. However, the mechanisms by which Lck and Fyn regulate TCR signaling are still not completely understood. One important question is whether Lck and Fyn have specific targets or only provide functional redundancy during TCR signaling. We have previously shown that Lck plays a major role in the tyrosine phosphorylation of the TCR-zeta chain and the ZAP-70 PTK. In an effort to identify the targets that are specifically regulated by Fyn, we have studied the tyrosine phosphorylation of **Pyk2** , a recently discovered new member of the focal adhesion kinase family PTK. We demonstrated that **Pyk2** was rapidly tyrosine phosphorylated following TCR stimulation. TCR-induced tyrosine phosphorylation of **Pyk2** was selectively dependent on Fyn but not Lck. Moreover, in heterologous COS-7 cells, coexpression of **Pyk2** with Fyn but not Lck resulted in substantial increases in **Pyk2** tyrosine phosphorylation. The selective regulation of **Pyk2** tyrosine phosphorylation by Fyn in vivo correlated with the preferential phosphorylation of **Pyk2** by Fyn in vitro. Our results demonstrate that **Pyk2** is a specific target regulated by Fyn during TCR signaling.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Protein-Tyrosine Kinase--metabolism--ME; *Proto-Oncogene Proteins--metabolism--ME; *Receptors, Antigen, T-Cell--metabolism--ME; *Signal Transduction; *T-Lymphocytes--metabolism--ME; Animals; Gene Expression Regulation, Enzymologic; Lymphocyte Specific Protein Tyrosine Kinase p56(lck); Mice; Mice, Inbred C57BL; Phosphorylation; Protein Binding ; Substrate Specificity; Tyrosine--metabolism--ME

CAS Registry No.: 0 (Proto-Oncogene Proteins); 0 (Receptors, Antigen, T-Cell); 0 (proto-oncogene protein c-fyn); 55520-40-6 (Tyrosine)

Enzyme No.: EC 2.7.1.- (protein tyrosine kinase **PYK2**); EC 2.7.1.112 (Protein-Tyrosine Kinase); EC 2.7.1.37 (Lymphocyte Specific Protein Tyrosine Kinase p56(lck))

Record Date Created: 19970514

Record Date Completed: 19970514

13456422 PMID: 9139918

Characterization of a glucose-repressed pyruvate kinase (Pyk2p) in Saccharomyces cerevisiae that is catalytically insensitive to fructose-1,6-bisphosphate.

Boles E; Schulte F; Miosga T; Freidel K; Schluter E; Zimmermann F K; Hollenberg C P; Heinisch J J

Institut fur Mikrobiologie, Heinrich-Heine-Universitat, Dusseldorf, Germany. boles@uni-duesseldorf.de

Journal of bacteriology (UNITED STATES) May 1997, 179 (9) p2987-93, ISSN 0021-9193 Journal Code: 2985120R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We have characterized the gene YOR347c of Saccharomyces cerevisiae and shown that it encodes a second functional pyruvate kinase isoenzyme, Pyk2p. Overexpression of the YOR347c/ **PYK2** gene on a multicopy vector restored growth on glucose of a yeast pyruvate kinase 1 (pyk1) mutant strain and could completely substitute for the PYK1-encoded enzymatic activity. **PYK2** gene expression is subject to glucose repression. A **pyk2** deletion mutant had no obvious growth phenotypes under various conditions, but the growth defects of a pyk1 **pyk2** double-deletion strain were even more pronounced than those of a pyk1 single-mutation strain. Pyk2p is active without fructose-1,6-bisphosphate. However, overexpression of **PYK2** during growth on ethanol did not cause any of the deleterious effects expected from a futile cycling between pyruvate and phosphoenolpyruvate. The results indicate that the **PYK2** -encoded pyruvate kinase may be used under conditions of very low glycolytic flux.

Tags: Comparative Study; Support, Non-U.S. Gov't

Descriptors: *Fructosediphosphates--metabolism--ME; *Pyruvate Kinase --genetics--GE; *Pyruvate Kinase--metabolism--ME; *Saccharomyces cerevisiae--enzymology--EN; *Saccharomyces cerevisiae--genetics--GE; Allosteric Regulation; Amino Acid Sequence; Animals; Base Sequence; Ethanol --metabolism--ME; Gene Deletion; Genes, Fungal; Genotype; Glucose --metabolism--ME; Glycolysis; Isoenzymes--chemistry--CH; Isoenzymes --genetics--GE; Isoenzymes--metabolism--ME; Kidney--enzymology--EN; Kinetics; Liver--enzymology--EN; Molecular Sequence Data; Muscle, Skeletal --enzymology--EN; Oligodeoxyribonucleotides; Pyruvate Kinase--chemistry--CH ; Rats; Recombinant Fusion Proteins--biosynthesis--BI; Recombinant Fusion Proteins--chemistry--CH; Recombinant Fusion Proteins--metabolism--ME; Saccharomyces cerevisiae--physiology--PH; Sequence Homology, Amino Acid; Substrate Specificity; beta-Galactosidase--metabolism--ME

CAS Registry No.: 0 (Fructosediphosphates); 0 (Isoenzymes); 0 (Oligodeoxyribonucleotides); 0 (Recombinant Fusion Proteins); 488-69-7 (fructose-1,6-diphosphate); 50-99-7 (Glucose); 64-17-5 (Ethanol)

Enzyme No.: EC 2.7.1.40 (Pyruvate Kinase); EC 3.2.1.23 (beta-Galactosidase)

Record Date Created: 19970529

Record Date Completed: 19970529

3477840 PMID: 9162070

Paxillin is tyrosine-phosphorylated by and preferentially associates with the calcium-dependent tyrosine kinase in rat liver epithelial cells.

Li X; Earp H S

Department of Pharmacology and Medicine and the Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599-7295, USA.

Journal of biological chemistry (UNITED STATES) May 30 1997, 272 (22) p14341-8, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

We and others have recently cloned a non-receptor, calcium-dependent tyrosine kinase (CADTK; also known as **PYK2** , CAKbeta, and RAFTK) that shares both overall domain structure and 45% amino acid identity with p125(FAK). We have studied the signaling, activation, and potential function of these related enzymes in GN4 rat liver epithelial cells that express CADTK and p125(FAK) at roughly similar levels. p125(FAK) is nearly fully tyrosine-phosphorylated in resting GN4 cells. In contrast, while CADTK is not tyrosine-autophosphorylated in untreated cells, angiotensin II increases CADTK Tyr(P) by 5-10-fold. With regard to signaling, CADTK activation is correlated with stimulation of c-Jun N-terminal kinase and p70(S6K) pathways but not with the stimulation of mitogen-activated protein kinase or p90(RSK). In this report we assessed the contribution of CADTK and p125(FAK) to tyrosine phosphorylation of focal contact proteins. In adherent GN4 cells, the constitutive activity of p125(FAK) was correlated with basal paxillin, tensin, and p130(CAS) tyrosine phosphorylation. A rapid increase in the tyrosine phosphorylation of each protein was detected after treatment with angiotensin II or other agonists that stimulate CADTK; the prolonged 3-4-fold increase in paxillin tyrosine phosphorylation was the most substantial change. In the WB cell line that expresses 3-fold less CADTK than GN4 cell line agonist-dependent paxillin tyrosine phosphorylation is similarly reduced. Immunoprecipitation of CADTK from GN4 cells revealed CADTK. paxillin complexes that persisted in 500 mM NaCl but not in 0.1% SDS cell lysis buffer. The complexes were largely independent of the tyrosine phosphorylation state of either protein. Surprisingly, we did not detect p125(FAK).paxillin complexes in immunoprecipitates using either of two p125(FAK) antibodies. When CADTK and p125(FAK) were transiently overexpressed in 293(T) cells, both enzymes associated with paxillin, but the avidity of CADTK appeared to be greater. In addition, in transfected 293(T) cells, complexes between CADTK and another potential substrate, p130(CAS), were detected. In summary, in GN4 rat liver epithelial cells stimulation of CADTK was highly correlated with paxillin tyrosine phosphorylation; in addition, CADTK but not p125(FAK) was complexed to paxillin at detectable levels. This suggests that agonist-dependent cytoskeletal changes in epithelial cells might proceed, in part, by CADTK-dependent mechanisms.

Tags: Support, Non-U.S. Gov't

Descriptors: *Calcium--metabolism--ME; *Cytoskeletal

13484775 PMID: 9169474

Protein kinase C and protein kinase A inhibit calcium-dependent but not stress-dependent c-Jun N-terminal kinase activation in rat liver epithelial cells.

Li X; Yu H; Graves L M; Earp H S

Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina 27599, USA.

Journal of biological chemistry (UNITED STATES) Jun 6 1997, 272 (23) p14996-5002, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

In rat liver epithelial cells (GN4), angiotensin II (Ang II) and thapsigargin stimulate a novel calcium-dependent tyrosine kinase (CADTK) also known as **PYK2** , CAKbeta, or RAFTK. Activation of CADTK by a thapsigargin-dependent increase in intracellular calcium failed to stimulate the extracellular signal-regulated protein kinase pathway but was well correlated with a 30-50-fold activation of c-Jun N-terminal kinase (JNK). In contrast, Ang II, which increased both protein kinase C (PKC) activity and intracellular calcium, stimulated extracellular signal-regulated protein kinase but produced a smaller, less sustained, JNK activation than thapsigargin. 12-O-Tetradecanoylphorbol 13-acetate (TPA), which slowly activated CADTK, did not stimulate JNK. These findings suggest either that CADTK is not involved in JNK activation or PKC activation inhibits the CADTK to JNK pathway. A 1-min TPA pretreatment of GN4 cells inhibited thapsigargin-dependent JNK activation by 80-90%. In contrast, TPA did not inhibit the >50-fold JNK activation effected by anisomycin or UV. The consequence of PKC-dependent JNK inhibition was reflected in c-Jun and c-Fos mRNA induction following treatment with thapsigargin and Ang II. Thapsigargin, which only minimally induced c-Fos, produced a much greater and more prolonged c-Jun response than Ang II. Elevation of another intracellular second messenger, cAMP, for 5-15 min also inhibited calcium-dependent JNK activation by approximately 80-90% but likewise had no effect on the stress-dependent JNK pathway. In summary, two pathways stimulate JNK in cells expressing CADTK, a calcium-dependent pathway modifiable by PKC and cAMP-dependent protein kinase and a stress-activated pathway independent of CADTK, PKC, and cAMP-dependent protein kinase; the inhibition by PKC can ultimately alter gene expression initiated by a calcium signal.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Ca(2+)-Calmodulin Dependent Protein Kinase--metabolism--ME; *Cyclic AMP-Dependent Protein Kinases--metabolism--ME; *Liver--enzymology --EN; *Mitogen-Activated Protein Kinases; *Protein Kinases--metabolism--ME; *Thapsigargin--pharmacology--PD; 1-Methyl-3-isobutylxanthine--pharmacology --PD; Androstadienes--pharmacology--PD; Angiotensin II--pharmacology--PD; Animals; Ca(2+)-Calmodulin Dependent Protein Kinase --antagonists and inhibitors--AI; Cells, Cultured; Cyclic AMP--metabolism--ME; Enzyme Activation; Enzyme Inhibitors--pharmacology--PD; Epidermal Growth Factor --pharmacology--PD; Epithelial Cells; Epithelium--drug effects--DE; Epithelium--enzymology--EN; Forskolin--pharmacology--PD; Kinetics; Models, Biological; Polyenes--pharmacology--PD; Rats; Recombinant Proteins --pharmacology--PD; Signal Transduction; Sirolimus; Tetradecanoylphorbol Acetate--pharmacology--PD

13552112 PMID: 9235901

Ras-dependent mitogen-activated protein kinase activation by G protein-coupled receptors. Convergence of Gi- and Gq-mediated pathways on calcium/calmodulin, Pyk2 , and Src kinase.

Della Rocca G J; van Biesen T; Daaka Y; Luttrell D K; Luttrell L M; Lefkowitz R J

Howard Hughes Medical Institute and the Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710, USA.

Journal of biological chemistry (UNITED STATES) Aug 1 1997, 272 (31) p19125-32, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: HL16037; HL; NHLBI; T32GM-07171; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Many receptors that couple to heterotrimeric guanine-nucleotide binding proteins (G proteins) have been shown to mediate rapid activation of the mitogen-activated protein kinases Erk1 and Erk2. In different cell types, the signaling pathways employed appear to be a function of the available repertoire of receptors, G proteins, and effectors. In HEK-293 cells, stimulation of either alpha1B- or alpha2A-adrenergic receptors (ARs) leads to rapid 5-10-fold increases in Erk1/2 phosphorylation. Phosphorylation of Erk1/2 in response to stimulation of the alpha2A-AR is effectively attenuated by pretreatment with pertussis toxin or by coexpression of a Gbetagamma subunit complex sequestrant peptide (betaARK1ct) and dominant-negative mutants of Ras (N17-Ras), mSOS1 (SOS-Pro), and Raf (DeltaN-Raf). Erk1/2 phosphorylation in response to alpha1B-AR stimulation is also attenuated by coexpression of N17-Ras, SOS-Pro, or DeltaN-Raf, but not by coexpression of betaARK1ct or by pretreatment with pertussis toxin. The alpha1B- and alpha2A-AR signals are both blocked by phospholipase C inhibition, intracellular Ca2+ chelation, and inhibitors of protein-tyrosine kinases. Overexpression of a dominant-negative mutant of c-Src or of the negative regulator of c-Src function, Csk, results in attenuation of the alpha1B-AR- and alpha2A-AR-mediated Erk1/2 signals. Chemical inhibitors of calmodulin, but not of PKC, and overexpression of a dominant-negative mutant of the protein-tyrosine kinase **Pyk2** also attenuate mitogen-activated protein kinase phosphorylation after both alpha1B- and alpha2A-AR stimulation. Erk1/2 activation, then, proceeds via a common Ras-, calcium-, and tyrosine kinase-dependent pathway for both Gi- and Gq/11-coupled receptors. These results indicate that in HEK-293 cells, the Gbetagamma subunit-mediated alpha2A-AR- and the Galphaq/11-mediated alpha1B-AR-coupled Erk1/2 activation pathways converge at the level of phospholipase C. These data suggest that calcium-calmodulin plays a central role in the calcium-dependent regulation of tyrosine phosphorylation by G protein-coupled receptors in some systems.

13677437 PMID: 9362541

Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5.

Davis C B; Dikic I; Unutmaz D; Hill C M; Arthos J; Siani M A; Thompson D A; Schlessinger J; Littman D R

Division of Molecular Pathogenesis, Skirball Institute for Biomolecular Medicine, NYU Medical Center 10016, USA. davis@saturn.med.nyu.edu

Journal of experimental medicine (UNITED STATES) Nov 17 1997, 186 (10) p1793-8, ISSN 0022-1007 Journal Code: 2985109R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS; AIDS/HIV

Infection with HIV-1 requires expression of CD4 and the chemokine receptors CXCR4 or CCR5 at the target cell surface. Engagement of these receptors by the HIV-1 envelope glycoprotein is essential for membrane fusion, but may additionally activate intracellular signaling pathways. In this study, we demonstrate that chemokines and HIV-1 envelope glycoproteins from both T-tropic and macrophage-tropic strains rapidly induce tyrosine phosphorylation of the protein tyrosine kinase **Pyk2** . The response requires CXCR4 and CCR5 to be accessible on the cell surface. The results presented here provide the first evidence for activation of an intracellular signaling event that can initiate multiple signaling pathways as a consequence of contact between HIV-1 and chemokine receptors.

Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Gene Products, env--immunology--IM; *HIV-1--immunology--IM; *Receptors, CCR5--metabolism--ME; *Receptors, CXCR4--metabolism--ME; *Signal Transduction--immunology--IM; Antigens, CD4--metabolism--ME; Calcium--metabolism--ME; Cell Line; Gene Products, env--biosynthesis--BI; Gene Products, env--metabolism--ME; HIV Envelope Protein gp120--metabolism --ME; HIV Envelope Protein gp120--pharmacology--PD; HIV-1--metabolism--ME; HL-60 Cells; Mutation; Phosphorylation; Protein Binding--immunology--IM; Protein-Tyrosine Kinase--metabolism--ME; Receptors, CCR5--deficiency--DF; Receptors, CCR5--genetics--GE; Signal Transduction--genetics--GE; Tyrosine --metabolism--ME

CAS Registry No.: 0 (Antigens, CD4); 0 (Gene Products, env); 0 (HIV Envelope Protein gp120); 0 (Receptors, CCR5); 0 (Receptors, CXCR4); 55520-40-6 (Tyrosine); 7440-70-2 (Calcium)

Enzyme No.: EC 2.7.1.- (protein tyrosine kinase **PYK2**); EC 2.7.1.112 (Protein-Tyrosine Kinase)

12641573 PMID: 7763235

The role of protein kinase in human synovial fibroblast growth.

Migita K; Eguchi K; Tsukada T; Kawabe Y; Aoyagi T; Nagataki S

First Department of Internal Medicine, Nagasaki University School of Medicine, Japan.

Biochemical and biophysical research communications (UNITED STATES) May 25 1995, 210 (3) p1066-75, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The histological features of rheumatoid arthritis (RA) consist of overgrowth of synovial cells. Several growth factors that cause synovial hyperplasia have been identified in RA synovium. The basic-fibroblast growth factor (b-FGF), representing one of these growth factors, may play an important role in the pathogenesis of RA. We examined the b-FGF-mediated intracellular signal pathway involved in synovial cell growth. b-FGF-induced synovial cell growth was **inhibited** by protein tyrosine kinase (PTK) **inhibitors** , herbimycin A and genistein, but not by H7 that **inhibits** protein kinase C (PKC). Stimulation of synovial cells with b-FGF resulted in tyrosine phosphorylation of cellular proteins and MAP kinase activation. Our results also demonstrated that b-FGF-mediated activation of MAP kinase was **inhibited** by herbimycin A indicating that protein tyrosine kinase may be involved in the activation of MAP kinase in human synovial cells. However, **inhibition** of b-FGF-mediated MAP kinase activation by PKC downregulation did not occur.

Tags: Comparative Study; Human

Descriptors: ***Arthritis, Rheumatoid** --enzymology--EN; *Cell Cycle --physiology--PH; *Cell Division; *Fibroblast Growth Factor 2--pharmacology --PD; *Protein Kinases--metabolism--ME; *Synovial Membrane--enzymology--EN; *Synovial Membrane--pathology--PA; 1-(5-Isoquinolinesulfonyl)-2-methylpiperazine; Arthritis, Rheumatoid--pathology--PA; Ca(2+)-Calmodulin Dependent Protein Kinase--metabolism--ME; Cell Cycle--drug effects--DE; Cell Division --drug effects--DE; Cells, Cultured; Fibroblasts--enzymology--EN; Genistein ; Hyperplasia; Isoflavones--pharmacology--PD; Isoquinolines--pharmacology --PD; Kinetics; Piperazines--pharmacology--PD; Protein Kinase C --antagonists and inhibitors--AI; Protein-Tyrosine Kinase-- **antagonists** and **inhibitors** --AI; Quinones--pharmacology--PD; **Signal Transduction** --drug effects--DE; **Signal Transduction** --physiology--PH; Thymidine --metabolism--ME

13612180 PMID: 9300723

Increased **expression of signaling lymphocytic activation molecule in patients with rheumatoid arthritis and its role in the** regulation **of cytokine production in rheumatoid synovium.**

Isomaki P; Aversa G; Cocks B G; Luukkainen R; Saario R; Toivanen P; de Vries J E; Punnonen J

Turku Immunology Centre/Department of Medical Microbiology, Turku University, Finland. pia.isomaki@utu.fi

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Sep 15 1997, 159 (6) p2986-93, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

In the present study the expression and function of signaling lymphocytic activation molecule (SLAM) in lymphocytes from patients with rheumatoid arthritis (RA) were investigated. The expression levels of SLAM were significantly up- **regulated** on synovial fluid and synovial tissue T cells from patients with RA compared with peripheral blood T cells from the same patients or from healthy volunteers. In addition, the expression of SLAM on peripheral blood B cells from patients with RA was elevated compared with that in healthy volunteers. SLAM+ T cells in synovial fluid coexpressed CD45RO and demonstrated **decreased** expression of CD27, indicative of a primed phenotype. In addition, the activation state of SLAM+ T cells was **enhanced** , as judged by **increased** expression of CD25, CD28, CD69, and CD95 on these cells. Interestingly, SLAM expression on activated CD4+ and CD8+ T cells from both patients and healthy individuals could be down- **regulated** by IL-10, which has been previously shown to function as an anti-inflammatory molecule in rheumatoid synovium. Furthermore, anti-SLAM mAbs **increased** the production of IL-10, IFN-gamma, and TNF-alpha by in vitro activated synovial fluid mononuclear cells, supporting the idea that signaling through SLAM may play a role in the **regulation** of synovial inflammation in patients with RA. Given the fact that SLAM was recently shown to be a high affinity self ligand, our data suggest that synovial T cells may stimulate their own cytokine production through homophilic SLAM-SLAM interactions.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Arthritis, Rheumatoid--immunology--IM; *Cytokines --biosynthesis--BI; *Glycoproteins--biosynthesis--BI; *Immunoglobulins --biosynthesis--BI; * **Signal Transduction** --immunology--IM; *Synovial Membrane--immunology--IM; Adult; Aged; Aged, 80 and over; Antigens, CD --biosynthesis--BI; Cytokines--immunology--IM; Flow Cytometry; Glycoproteins--immunology--IM; Immunoglobulins--immunology--IM; Immunophenotyping; Lymphocyte Activation; Middle Aged

CAS Registry No.: 0 (Antigens, CD); 0 (Cytokines); 0 (Glycoproteins); 0 (Immunoglobulins); 169535-43-7 (SLAM protein)

Record Date Created: 19971008

Record Date Completed: 19971008

09331718 PMID: 1351318

Tyrosine kinase **signal transduction in rheumatoid synovitis.**

Williams W V; VonFeldt J M; Ramanujam T; Weiner D B

Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia.

Seminars in arthritis and rheumatism (UNITED STATES) Apr 1992, 21 (5) p317-29, ISSN 0049-0172 Journal Code: 1306053

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Explants of synovial cells in rheumatoid arthritis display a transformed phenotype with focus formation and anchorage-independent growth. Many of the cytokines that activate these fibroblasts mediate their action through tyrosine **kinase** growth factor receptors. Mechanisms of signal transduction via such tyrosine **kinases** are therefore relevant to the pathogenesis of rheumatoid lesions. Data are presented using the neu oncogene product p185neu as a model system to explore signal transduction by receptor tyrosine **kinases** . Evidence is shown that **increased** tyrosine **kinase** activity in the oncogenic form of this protein may result from dimerization of the tyrosine **kinase** receptor. In the normal cellular counterpart of p185neu, dimerization appears to be mediated by the action of an as yet unidentified ligand. Dimerization also appears to be important in signal transduction mediated by epidermal growth factor, platelet-derived growth factor, and colony-stimulating factor 1. These cytokines also **alter** the phenotype of rheumatoid synovial fibroblasts to resemble transformed fibroblasts. Additionally, preliminary data that suggest **increased** tyrosine **kinase** activity in rheumatoid arthritis synovia compared with osteoarthritis synovia are presented. Molecular characterization of tyrosine **kinase** receptors will be an important direction for future studies of the pathogenesis of rheumatoid disease.

Tags: Comparative Study; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Protein-Tyrosine **Kinase** --physiology--PH; * **Signal Transduction** --physiology--PH; *Synovitis--physiopathology--PP; Animals; **Arthritis, Rheumatoid** --enzymology--EN; Cells, Cultured; Electrophoresis, Polyacrylamide Gel; Fibroblasts--cytology--CY; Fibroblasts--physiology--PH ; Fibroblasts--ultrastructure--UL; Mice; Neuroblastoma--enzymology--EN; Neuroblastoma--pathology--PA; Neuroblastoma--ultrastructure--UL; Osteoarthritis--enzymology--EN; Precipitin Tests; Protein-Tyrosine **Kinase** --analysis--AN; Protein-Tyrosine **Kinase** --chemistry--CH; Proto-Oncogene Proteins--analysis--AN; Proto-Oncogene Proteins--physiology--PH; Rats; Receptor, erbB-2; Receptors, Cell Surface--analysis--AN; Receptors, Cell Surface--chemistry--CH; Receptors, Cell Surface--physiology--PH; Synovial Membrane--cytology--CY; Synovial Membrane--drug effects--DE; Synovial Membrane--enzymology--EN; Tumor Cells, Cultured--enzymology--EN; Tumor Cells, Cultured--pathology--PA; Tumor Cells, Cultured--ultrastructure--UL

CAS Registry No.: 0 (Proto-Oncogene Proteins); 0 (Receptors, Cell Surface)

Enzyme No.: EC 2.7.1.112 (Protein-Tyrosine **Kinase**); EC 2.7.1.112 (Receptor, erbB-2)

Record Date Created: 19920713

Record Date Completed: 19920713

12641573 PMID: 7763235

The role of protein kinase **in human synovial fibroblast growth.**

Migita K; Eguchi K; Tsukada T; Kawabe Y; Aoyagi T; Nagataki S

First Department of Internal Medicine, Nagasaki University School of Medicine, Japan.

Biochemical and biophysical research communications (UNITED STATES) May 25 1995, 210 (3) p1066-75, ISSN 0006-291X Journal Code: 0372516

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The histological features of rheumatoid arthritis (RA) consist of overgrowth of synovial cells. Several growth factors that cause synovial hyperplasia have been identified in RA synovium. The basic-fibroblast growth factor (b-FGF), representing one of these growth factors, may play an important role in the pathogenesis of RA. We examined the b-FGF-mediated intracellular signal pathway involved in synovial cell growth. b-FGF-induced synovial cell growth was **inhibited** by protein tyrosine **kinase** (PTK) **inhibitors** , herbimycin A and genistein, but not by H7 that **inhibits** protein **kinase** C (PKC). Stimulation of synovial cells with b-FGF resulted in tyrosine **phosphorylation** of cellular proteins and MAP **kinase** activation. Our results also demonstrated that b-FGF-mediated activation of MAP **kinase** was **inhibited** by herbimycin A indicating that protein tyrosine **kinase** may be involved in the activation of MAP **kinase** in human synovial cells. However, **inhibition** of b-FGF-mediated MAP **kinase** activation by PKC downregulation did not occur.

Tags: Comparative Study; Human

Descriptors: ***Arthritis, Rheumatoid** --enzymology--EN; *Cell Cycle --physiology--PH; *Cell Division; *Fibroblast Growth Factor 2--pharmacology --PD; *Protein **Kinases** --metabolism--ME; *Synovial Membrane--enzymology --EN; *Synovial Membrane--pathology--PA; 1-(5-Isoquinolinesulfonyl)-2-meth ylpiperazine; Arthritis, Rheumatoid--pathology--PA; Ca(2+)-Calmodulin Dependent Protein **Kinase** --metabolism--ME; Cell Cycle--drug effects--DE; Cell Division--drug effects--DE; Cells, Cultured; Fibroblasts--enzymology --EN; Genistein; Hyperplasia; Isoflavones--pharmacology--PD; Isoquinolines --pharmacology--PD; Kinetics; Piperazines--pharmacology--PD; Protein **Kinase** C--antagonists and inhibitors--AI; Protein-Tyrosine **Kinase** -- **antagonists** and **inhibitors** --AI; Quinones--pharmacology--PD; **Signal Transduction** --drug effects--DE; **Signal Transduction** --physiology--PH; Thymidine--metabolism--ME

CAS Registry No.: 0 (Isoflavones); 0 (Isoquinolines); 0 (Piperazines); 0 (Quinones); 103107-01-3 (Fibroblast Growth Factor 2); 446-72-0 (Genistein); 50-89-5 (Thymidine); 70563-58-5 (herbimycin); 84477-87-2 (1-(5-Isoquinolinesulfonyl)-2-methylpiperazine)

Enzyme No.: EC 2.7.1.112 (Protein-Tyrosine **Kinase**); EC 2.7.1.123 (Ca(2+)-Calmodulin Dependent Protein **Kinase**); EC 2.7.1.37 (Protein **Kinase** C); EC 2.7.1.37 (Protein **Kinases**)

Record Date Created: 19950629

Record Date Completed: 19950629

10331949 PMID: 7530065

Signal transduction in T lymphocytes of SLE patients: lectin-activated phosphorylation **on tyrosine.**

Matache C; Stefanescu M; Onu A; Muresan D; Szegli G

Cantacuzino Institute, Bucharest, Romania.

Roumanian archives of microbiology and immunology (ROMANIA) Oct-Dec 1993, 52 (4) p277-84, ISSN 1222-3891 Journal Code: 9204717

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A comparative study of signal transduction through tyrosine **phosphorylation** process in peripheral blood lymphocytes from SLE patients and healthy subjects reveal some modifications in the **phosphorylation** pattern of SLE T lymphocytes. Thus, the level of constitutive tyrosine **phosphorylation** in resting SLE T lymphocytes is higher than in lymphocytes from healthy subjects. In SLE T lymphocytes, a cellular proteic substrate with an apparent molecular weight of about 37 kDa is constitutively **phosphorylated** . Some differences in the pattern of **phosphorylation** are obvious in lectin (Con A, PHA)-activated T lymphocytes. Thus, Con A activation **enhances** the **phosphorylation** of cellular substrates with molecular weight in the range of 55-80 kDa from SLE T lymphocytes. Moreover, the 21 kDa substrate is also hyperphosphorylated after PHA activation of SLE lymphocytes.

Tags: Comparative Study; Human

Descriptors: *Autoimmune Diseases--blood--BL; *Lectins--pharmacology--PD; * **Lupus Erythematosus, Systemic** --blood--BL; * **Signal Transduction** --drug effects--DE; *T-Lymphocytes--drug effects--DE; *Tyrosine--drug effects--DE ; Autoradiography; Cell Separation; Phosphopeptides--blood--BL; Phosphopeptides--isolation and purification--IP; **Phosphorylation** --drug effects--DE; Phosphotyrosine; Precipitin Tests; T-Lymphocytes--metabolism --ME; Tyrosine--analogs and derivatives--AA; Tyrosine--blood--BL; Tyrosine--isolation and purification--IP

CAS Registry No.: 0 (Lectins); 0 (Phosphopeptides); 21820-51-9 (Phosphotyrosine); 55520-40-6 (Tyrosine)

Record Date Created: 19950222

Record Date Completed: 19950222

12929428 PMID: 8603539

The effects of the immunosuppressant rapamycin on the growth of rheumatoid arthritis (RA) synovial fibroblast.

Migita K; Eguchi K; aoyagi T; Tsukada T; Tsuboi M; Kawabe Y; Nagataki S

First Department of Internal Medicine, Nagasaki University School of Medicine, Japan.

Clinical and experimental immunology (ENGLAND) Apr 1996, 104 (1) p86-91, ISSN 0009-9104 Journal Code: 0057202

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

RA is a chronic inflammatory disease characterized by mononuclear cell infiltration and the overgrowth of synovial fibroblast. This invasive growth of synovial tissues corresponds with the progressive destruction of articular cartilage and bone. Several immunosuppressive agents, such as cyclophosphamide, cyclosporin A and mizoribine, have been clinically used to control disease progression, though relatively little is known of their effects on rheumatoid synovium. Rapamycin exhibits a strong immunosuppressive activity by acting on T cell signalling pathways. In the present study we examined the effects of rapamycin on the growth of synovial fibroblast isolated from RA patients. Platelet-derived growth factor (PDGF) is a potent growth factor in synovial fibroblasts isolated from RA patients. PDGF and serum stimulation resulted in a rapid **phosphorylation** of tyrosine and activation of mitogen-activated protein **kinase** (MAP **kinase**), 70-kD-S6 **kinase** (P70S6k) and 90-kD-S6 **kinase** (P90rsk). Rapamycin, a macrolide immunosuppressant, **inhibited** completely growth factor-induced synovial fibroblast proliferation and P70S6k activation. In contrast, tyrosine **phosphorylation** and activation of MAP **kinases** and P90rsk were not influenced by rapamycin treatment. Our data demonstrate that growth factor-mediated P70S6k activation is closely related to the growth of synovial fibroblast, and suggest the efficacy of rapamycin for controlling synovial hyperplasia in RA.

Tags: Human

Descriptors: ***Arthritis, Rheumatoid** --immunology--IM; *Immunosuppressive Agents--pharmacology--PD; *Polyenes--pharmacology--PD; *Synovial Membrane --cytology--CY; **Arthritis, Rheumatoid** --pathology--PA; Ca(2+)-Calmodulin Dependent Protein **Kinase** --metabolism--ME; Cell Cycle--drug effects--DE; Cell Division--drug effects--DE; Cells, Cultured; Enzyme Activation; Fibroblasts--cytology--CY; Phosphotyrosine--metabolism--ME; Platelet-Derived Growth Factor--pharmacology--PD; Protein-Serine-Threonine **Kinases** --metabolism--ME; Receptors, Platelet-Derived Growth Factor--physiology--PH ; Ribosomal Protein S6 **Kinases** ; **Signal** Transduction; Sirolimus

CAS Registry No.: 0 (Immunosuppressive Agents); 0 (Platelet-Derived Growth Factor); 0 (Polyenes); 21820-51-9 (Phosphotyrosine); 53123-88-9 (Sirolimus)

Enzyme No.: EC 2.7.1.112 (Receptors, Platelet-Derived Growth Factor); EC 2.7.1.123 (Ca(2+)-Calmodulin Dependent Protein **Kinase**); EC 2.7.1.37 (Protein-Serine-Threonine **Kinases**); EC 2.7.1.37 (Ribosomal Protein S6 **Kinases**)

Record Date Created: 19960515

Record Date Completed: 19960515

13292711 PMID: 8958217

B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events.

Liossis S N; Kovacs B; Dennis G; Kammer G M; Tsokos G C

Department of Clinical Physiology, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA.

Journal of clinical investigation (UNITED STATES) Dec 1 1996, 98 (11) p2549-57, ISSN 0021-9738 Journal Code: 7802877

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

To understand the molecular mechanisms that are responsible for the B cell overactivity that is observed in patients with SLE, we have conducted experiments in which the surface immunoglobulin (sIg)-mediated early cell signaling events were studied. The anti-sIgM-mediated free intracytoplasmic calcium ([Ca2+]i) responses were significantly higher in SLE B cells compared with responses of normal individuals and to those of patients with other systemic autoimmune rheumatic diseases. The anti-IgD mAb induced [Ca2+]i responses were also higher in lupus B cells than in controls. The magnitude of anti-sIgM-mediated Ca2+ release from intracellular stores was also **increased** in B cells from SLE patients compared with normal controls. The amount of inositol phosphate metabolites produced upon crosslinking of sIgM was slightly higher in patients with lupus than in normal controls, although the difference was not statistically significant. In contrast, the degree of anti-sIgM-induced protein tyrosine **phosphorylation** was obviously **increased** in lupus patients. Our study demonstrates clearly for the first time that SLE B cells exhibit aberrant early signal transduction events, including augmented calcium responses after crosslinking of the B cell receptor and **increased** antigen-receptor-mediated **phosphorylation** of protein tyrosine residues. Because the above abnormalities did not correlate with disease activity or treatment status, we propose that they may have pathogenic significance.

Tags: Female; Human; Male; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: *B-Lymphocytes--physiology--PH; * **Lupus Erythematosus, Systemic** --immunology--IM; *Receptors, Antigen, B-Cell--physiology--PH; * **Signal Transduction** ; Adult; Aged; Antibodies, Monoclonal--pharmacology --PD; B-Lymphocytes--immunology--IM; Calcium--blood--BL; Cells, Cultured; Cytoplasm--metabolism--ME; Flow Cytometry; Immunoglobulin M--immunology--IM ; Immunoglobulin M--physiology--PH; Inositol Phosphates--metabolism--ME; Kinetics; **Lupus Erythematosus, Systemic** --blood--BL; Middle Aged; Reference Values

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Immunoglobulin M); 0 (Inositol Phosphates); 0 (Receptors, Antigen, B-Cell); 7440-70-2 (Calcium)

Record Date Created: 19970123

Record Date Completed: 19970123

3124400 PMID: 8792832

Precise localization of the human gene encoding cell adhesion kinase beta (CAK beta/ PYK2) to chromosome 8 at p21.1 by fluorescence in situ hybridization.

Inazawa J; Sasaki H; Nagura K; Kakazu N; Abe T; Sasaki T

Department of Hygiene, Kyoto Prefectural University of Medicine, Japan.

Human genetics (GERMANY) Oct 1996, 98 (4) p508-10, ISSN 0340-6717 Journal Code: 7613873

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Cell adhesion kinase beta (CAK beta) is the second protein-tyrosine kinase of the focal adhesion kinase subfamily with large N- and C-domains in addition to the central kinase domain. The cDNA of the human CAK beta has been cloned and used as a probe for the assignment of this gene by fluorescence in situ hybridization. CAK beta is sublocalized on chromosome 8p21.1, a locus frequently involved in allelic losses in colorectal cancers and prostate carcinomas.

Tags: Human; Male; Support, Non-U.S. Gov't

Descriptors: *Chromosomes, Human, Pair 8; *Protein-Tyrosine Kinase --genetics--GE; Animals; Chromosome Mapping; Colorectal Neoplasms--genetics --GE; DNA Probes; DNA, Complementary; In Situ Hybridization, Fluorescence; Karyotyping; Molecular Sequence Data; Prostatic Neoplasms--genetics--GE; Rats

Molecular Sequence Databank No.: GENBANK/L49207; GENBANK/U43522

CAS Registry No.: 0 (DNA Probes); 0 (DNA, Complementary)

Enzyme No.: EC 2.7.1.- (protein tyrosine kinase **PYK2**); EC 2.7.1.112 (Protein-Tyrosine Kinase)

Record Date Created: 19961015

Record Date Completed: 19961015

13270782 PMID: 8939945

Activation of a novel calcium-dependent protein-tyrosine kinase. Correlation with c-Jun N-terminal kinase but not mitogen-activated protein kinase activation.

Yu H; Li X; Marchetto G S; Dy R; Hunter D; Calvo B; Dawson T L; Wilm M; Anderegg R J; Graves L M; Earp H S

Department of Medicine, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina 27599, USA.

Journal of biological chemistry (UNITED STATES) Nov 22 1996, 271 (47) p29993-8, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: DK 31683; DK; NIDDK

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Many G protein-coupled receptors (e.g. that of angiotensin II) activate phospholipase Cbeta, initially increasing intracellular calcium and activating protein kinase C. In the WB and GN4 rat liver epithelial cell lines, agonist-induced calcium signals also stimulate tyrosine phosphorylation and subsequently increase the activity of c-Jun N-terminal kinase (JNK). We have now purified the major calcium-dependent tyrosine kinase (CADTK), and by peptide and nucleic acid sequencing identified it as a rat homologue of human **PYK2** . CADTK/ **PYK2** is most closely related to p125(FAK) and both enzymes are expressed in WB and GN4 cells. Angiotensin II, which only slightly increases p125(FAK) tyrosine phosphorylation in GN4 cells, substantially increased CADTK tyrosine autophosphorylation and kinase activity. Agonists for other G protein-coupled receptors (e.g. LPA), or those increasing intracellular calcium (thapsigargin), also stimulated CADTK. In comparing the two rat liver cell lines, GN4 cells exhibited approximately 5-fold greater angiotensin II- and thapsigargin-dependent CADTK activation than WB cells. Although maximal JNK activation by stress-dependent pathways (e.g. UV and anisomycin) was equivalent in the two cell lines, calcium-dependent JNK activation was 5-fold greater in GN4, correlating with CADTK activation. In contrast to JNK, the thapsigargin-dependent calcium signal did not activate mitogen-activated protein kinase and Ang II-dependent mitogen-activated protein kinase activation was not correlated with CADTK activation. Finally, while some stress-dependent activators of the JNK pathway (NaCl and sorbitol) stimulated CADTK, others (anisomycin, UV, and TNFalpha) did not. In summary, cells expressing CADTK/ **PYK2** appear to have two alternative JNK activation pathways: one stress-activated and the other calcium-dependent.

Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Ca(2+)-Calmodulin Dependent Protein Kinase--metabolism--ME; *Calcium--metabolism--ME; *Mitogen-Activated Protein Kinases; *Mitogens --pharmacology--PD; *Protein-Tyrosine Kinase--metabolism--ME; Amino Acid Sequence; Animals; Cell Line; DNA, Complementary; Enzyme Activation; Molecular Sequence Data; Rats

Molecular Sequence Databank No.: GENBANK/U69109

CAS Registry No.: 0 (DNA, Complementary); 0 (Mitogens); 7440-70-2 (Calcium)

Enzyme No.: EC 2.7.1.112 (Protein-Tyrosine Kinase); EC 2.7.1.123 (Ca(2+)-Calmodulin Dependent Protein Kinase); EC 2.7.1.37 (Mitogen-Activated Protein Kinases); EC 2.7.10.- (c-Jun amino-terminal kinase)

Record Date Created: 19970113

Record Date Completed: 19970113

13348677 PMID: 9022036

Cytotoxic T lymphocytes express a beta3 integrin which can induce the phosphorylation of focal adhesion kinase and the related PYK-2.

Ma E A; Lou O; Berg N N; Ostergaard H L

Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada.

European journal of immunology (GERMANY) Jan 1997, 27 (1) p329-35, ISSN 0014-2980 Journal Code: 1273201

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Fibronectin has been shown to stimulate tyrosine phosphorylation of a number of proteins in the 115-125 kDa range and facilitate degranulation by alloantigen-specific cytotoxic T lymphocyte (CTL) clones in response to substimulatory amounts of anti-CD3 or anti-T cell receptor (TCR). The current study was initiated to further characterize integrin expression and usage by these CTL clones. We demonstrate that vitronectin and fibrinogen, but not laminin or collagen, are also able to both facilitate degranulation in the presence of substimulatory anti-CD3 and stimulate tyrosine phosphorylation of these 115-125-kDa proteins, with a 115-kDa protein being the most prominently phosphorylated. These results implicate the expression and usage of the vitronectin receptor, alpha beta3 integrin, by these CTL clones. We demonstrate by both flow cytometry and immunoprecipitation that CTL clones do in fact express beta3 integrin. Immobilized antibody to beta3 stimulates the phosphorylation of the 115-125-kDa proteins, suggesting that engagement of beta3 transmits the same signal into these cells as fibronectin or vitronectin. The fibronectin and vitronectin-induced phosphorylation as well as adhesion to either fibronectin or vitronectin can be significantly inhibited with antibodies to beta3 integrins. Finally, we are able to immunoprecipitate 115-kDa proteins with antiserum to focal adhesion kinase and a related kinase, called PYK-2, that becomes phosphorylated in response to vitronectin or immobilized anti-beta3. Taken together, these results demonstrate that CTL express and use beta3-integrins as signaling molecules which can augment TCR-mediated stimulation.

Tags: Support, Non-U.S. Gov't

Descriptors: *Antigens, CD--metabolism--ME; *Cell Adhesion Molecules --metabolism--ME; *Lymphocyte Activation; *Platelet Membrane Glycoproteins --metabolism--ME; *Protein-Tyrosine Kinase--metabolism--ME; *T-Lymphocytes, Cytotoxic--metabolism--ME; Animals; Cell Adhesion; Cell Degranulation; Clone Cells; Fibrinogen--metabolism--ME; Fibronectins--metabolism--ME; Immunophenotyping; Integrin beta3; Mice; Molecular Weight; Phosphoproteins --chemistry--CH; Phosphoproteins--metabolism--ME; Phosphorylation; Phosphotyrosine--metabolism--ME; Signal Transduction; Vitronectin --metabolism--ME

13612178 PMID: 9300721

Defective TCR-mediated signaling in synovial T cells in rheumatoid arthritis.

Maurice M M; Lankester A C; Bezemer A C; Geertsma M F; Tak P P; Breedveld F C; van Lier R A; Verweij C L

Department of Rheumatology, Leiden University Hospital, The Netherlands.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Sep 15 1997, 159 (6) p2973-8, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

In rheumatoid arthritis (RA), the functional status of T cells is incompletely understood. Synovial T cells display phenotypic evidence of former activation, but there is poor production of T cell-derived cytokines in the synovium. In addition, synovial T cell proliferation upon mitogenic and antigenic stimulation was **decreased** compared with that in peripheral blood T cells. Moreover, previous reports revealed that early Ca2+ rises induced by TCR/CD3 stimulation were **decreased** in RA T cells compared with those in healthy controls. To investigate the molecular mechanisms of RA synovial T cell hyporesponsiveness, we analyzed the TCR/CD3-mediated protein tyrosine **phosphorylation** in RA peripheral blood and synovial fluid (SF) T cells. SF T cells exhibited a **decreased** overall tyrosine **phosphorylation** pattern upon stimulation. Most notably, the induction of **phosphorylation** of p38 was virtually absent. Moreover, we found that tyrosine **phosphorylation** of the TCR zeta-chain, one of the most proximal events in TCR signaling, is clearly diminished in RA SF T cells. The **decrease** in tyrosine **phosphorylation** was accompanied by a **decrease** in detectable levels of zeta-protein within synovial T cells. These results suggest that a defective TCR signaling underlies the hyporesponsiveness of synovial T cells in RA.

Tags: Human

Descriptors: ***Arthritis, Rheumatoid** --immunology--IM; *Membrane Proteins --immunology--IM; *Receptors, Antigen, T-Cell--immunology--IM; * **Signal Transduction** --immunology--IM; *Synovial Membrane--immunology--IM; *T-Lymphocytes--immunology--IM; Cells, Cultured; Flow Cytometry; Immunohistochemistry

CAS Registry No.: 0 (Membrane Proteins); 0 (Receptors, Antigen, T-Cell); 0 (antigen T cell receptor, zeta chain)

Record Date Created: 19971008

Record Date Completed: 19971008

13750103 PMID: 9444427

Immune cell biochemical abnormalities in systemic lupus erythematosus.

Liossis S N; Vassilopoulos D; Kovacs B; Tsokos G C

Department of Clinical Physiology, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA.

Clinical and experimental rheumatology (ITALY) Nov-Dec 1997, 15 (6) p677-84, ISSN 0392-856X Journal Code: 8308521

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Novel data have emerged which attempt to characterize the biochemical abnormalities that are exhibited by lupus immune cells. Lupus lymphocytes display abnormal antigen-receptor-mediated signaling, consisting of **increased** Ca2+ mobilization and **increased** protein tyrosyl **phosphorylation** that are independent of disease activity. Abnormalities in the expression and function of co-stimulatory molecules (B7-CD28 and CD40-CD40L) have been established. Transcription of cytokine genes and the methylation of DNA which affects multiple genes are also abnormal. Finally, aberrations of the apoptosis of lupus immune cells are contributors to the pathogenesis of the disease. (48 Refs.)

Tags: Human

Descriptors: *B-Lymphocytes--immunology--IM; * **Lupus Erythematosus, Systemic** --immunology--IM; * **Signal Transduction** --immunology--IM; *T-Lymphocytes--immunology--IM; B-Lymphocytes--chemistry--CH; T-Lymphocytes --chemistry--CH

Record Date Created: 19980303

Record Date Completed: 19980303

2752035 PMID: 7673154

Cloning and characterization of cell adhesion kinase beta, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily.

Sasaki H; Nagura K; Ishino M; Tobioka H; Kotani K; Sasaki T

Department of Biochemistry, Sapporo Medical University School of Medicine, Japan.

Journal of biological chemistry (UNITED STATES) Sep 8 1995, 270 (36) p21206-19, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A second protein-tyrosine kinase (PTK) of the focal adhesion kinase (FAK) subfamily, cell adhesion kinase beta (CAK beta), was identified by cDNA cloning. The rat CAK beta is a 115.7-kDa PTK that contains N- and C-terminal domains of 418 and 330 amino acid residues besides the central kinase domain. The rat CAK beta has a homology with mouse FAK over their entire lengths except for the extreme N-terminal 88 residues and shares 45% overall sequence identity (60% identical in the catalytic domain), which indicates that CAK beta is a protein structurally related to but different from FAK. The CAK beta gene is less evenly expressed in a variety of rat organs than the FAK gene. Anti-CAK beta antibody immunoprecipitated a 113-kDa protein from rat brain, 3Y1 fibroblasts, and COS-7 cells transfected with CAK beta cDNA. The tyrosine-phosphorylated state of CAK beta was not reduced on trypsinization, nor enhanced in response to plating 3Y1 cells onto fibronectin. CAK beta localized to sites of cell-to-cell contact in COS-7 transfected with CAK beta cDNA, in which FAK was found at the bottom of the cells. Thus, CAK beta is a PTK possibly participating in the signal transduction regulated by cell-to-cell contacts.

Tags: Human

Descriptors: *Cell Adhesion Molecules--genetics--GE; *Protein-Tyrosine Kinase--genetics--GE; Amino Acid Sequence; Animals; Base Sequence; Brain --enzymology--EN; Cell Adhesion Molecules--metabolism--ME; Cell Line; Cloning, Molecular; DNA, Complementary; Molecular Sequence Data; Phosphorylation; Protein-Tyrosine Kinase--metabolism--ME; RNA, Messenger --genetics--GE; RNA, Messenger--metabolism--ME; Sequence Homology, Amino Acid; Tumor Cells, Cultured; Tyrosine--metabolism--ME

Molecular Sequence Databank No.: GENBANK/D45853; GENBANK/D45854

CAS Registry No.: 0 (Cell Adhesion Molecules); 0 (DNA, Complementary) ; 0 (RNA, Messenger); 55520-40-6 (Tyrosine)

Enzyme No.: EC 2.7.1.- (endogenous substrate pp120); EC 2.7.1.- (protein tyrosine kinase **PYK2**); EC 2.7.1.112 (Protein-Tyrosine Kinase)

Gene Symbol: CAK beta ; FAK

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S2 3137 PYK2?
S3 146572 PROTEIN (N) TYROSINE? (2N) KINASE?
S4 148375 S1 OR S2 OR S3
S5 3027 S4 AND (DISEASE? OR ILLNESS? OR DISORDER? OR CORRELAT? OR -PATHOLOG?)/TI
S6 1728 RD (unique items)
S7 1570 S5/1998:2005
S8 1299 S5 NOT S6
S9 842 S6 NOT S7
S10 292 S9 AND (ASSAY? OR IMMUNOASSAY? OR DETERM? OR DETECT? OR MEASURE?)
S11 253 S9 AND (NORMAL? OR CONTROL? OR REFERENCE?)
S12 115 (S10 OR S11) AND (SIGNAL? (3N) TRANSDUC?)
S13 50 TARGET - S12
?t s13/9/41 42 46 8 10 13 14 17 27 29 33 39

13/9/41 (Item 41 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03077829 Genuine Article#: NA555 Number of References: 33
Title: FIBROBLAST EXPRESSION OF COLLAGENS AND PROTEOGLYCANS IS ALTERED IN ASPARTYLGLUCOSAMINURIA, A LYSOSOMAL STORAGE DISEASE
Author(s): MAATTA A; JARVELAINEN HT; NELIMARKKA LO; PENTTINEN RP
Corporate Source: UNIV TURKU,DEPT MED BIOCHEM/SF-20520 TURKU//FINLAND/; UNIV TURKU,DEPT MED BIOCHEM/SF-20520 TURKU//FINLAND/
Journal: BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE, 1994, V 1225, N3 (FEB 22), P264-270
ISSN: 0925-4439
Language: ENGLISH Document Type: ARTICLE
Geographic Location: FINLAND
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; BIOPHYSICS
Abstract: Aspartylglucosaminuria (AGU), a lysosomal storage disease caused by deficient activity of aspartulglucosaminidase (E.C. 3.5.1.26), is characterised by progressive mental retardation and variable connective tissue signs. The ultrastructure of collagen fibrils in skin of AGU patients is abnormal and their fibroblasts synthesise reduced amounts of collagens [Nanto-Salonen et al. (1984) Lab invest. 51: 464-468]. In this work we measured the steady-state messenger RNA levels of several extracellular matrix components in skin fibroblast cultures of two patients homozygous for the most prevalent mutation (AGU,,,) causing the disease in Finland. In confluent cultures the steady-state mRNA concentrations of type I and III collagens were reduced to 0.5-20% of **control** values. Almost as marked reduction was observed in the mRNA level of biglycan, a small interstitial proteoglycan whereas that of decorin, a closely related, collagen fibril-associated proteoglycan, was increased several-fold. Elevated decorin and decreased biglycan mRNA levels reflected the amounts of the produced corresponding proteoglycans. The differences in the mRNA levels became more pronounced with the time the cells were in culture. Fibronectin mRNA concentrations were similar in AGU and **control** fibroblasts. Changes in the expression and synthesis of extracellular matrix components might be related to the connective tissue symptoms of the patients.
Descriptors--Author Keywords: GENE EXPRESSION ; COLLAGEN ; PROTEOGLYCAN ;

EXTRACELLULAR MATRIX ; LYSOSOME ; ASPARTYLGLUCOSAMINURIA
Identifiers--KeyWords Plus: GROWTH-FACTOR-BETA; MESSENGER-RNA; DIFFERENTIAL EXPRESSION; GENE-EXPRESSION; SMOOTH-MUSCLE; DECORIN; LOCALIZATION; BIGLYCAN; TISSUES; CELLS
Research Fronts: 92-0089 002 (BASIC FIBROBLAST GROWTH-FACTOR; TRANSMEMBRANE HEPARAN-SULFATE PROTEOGLYCANS; CYSTEINE-RICH RECEPTOR)
92-6678 001 (REGULATION OF THE VLA INTEGRIN LIGAND INTERACTIONS; FOCAL ADHESION-ASSOCIATED **PROTEIN TYROSINE KINASE** ; DIFFERENTIAL EXPRESSION; **SIGNAL TRANSDUCTION**)
Cited References:

AUTIO S, 1972, V1, P1, J MENT DEFIC RES
BROWN DC, 1989, V9, P468, MATRIX
CHOMCZYNSKI P, 1987, V162, P156, ANAL BIOCHEM
ENOMAA N, 1992, V286, P613, BIOCHEM J
FISCHER L, 1989, V264, P4571, J BIOL CHEM
FISHER LW, 1991, V266, P4371, J BIOL CHEM
FLEISCHMAJER R, 1991, V106, P82, J STRUCT BIOL
FOCHT RJ, 1984, V4, P1843, MOL CELL BIOL
FORT P, 1985, V13, P1431, NUCLEIC ACIDS RES
HYNES RO, 1992, V69, P11, CELL
IKONEN E, 1991, V88, P1222, P NATL ACAD SCI USA
IRUELAARISPE ML, 1991, V64, P174, LAB INVEST
JARVELAINEN HT, 1991, V266, P3274, J BIOL CHEM
KAHARI VM, 1991, V266, P608, J BIOL CHEM
KIVIRIKKO KI, 1979, V8, P23, INT REV CONNECT TISS
KRUSIUS T, 1986, V83, P7683, P NATL ACAD SCI USA
LARK MW, 1986, V6, P638, ARTERIOSCLEROSIS
LIAU G, 1985, V260, P531, J BIOL CHEM
MAKELA JK, V16, P349, NUCLEIC ACIDS RES
NANTOSALONEN K, 1987, V16, P367, CONNECT TISSUE RES
NANTOSALONEN K, 1982, V5, P197, J INHERIT METAB DIS
NANTOSALONEN K, 1984, V51, P464, LAB INVEST
PENTTINEN RP, 1988, V85, P1105, P NATL ACAD SCI USA
PRINGLE GA, 1990, V38, P1401, J HISTOCHEM CYTOCHEM
RAPRAEGER A, 1985, V260, P4103, J BIOL CHEM
RUTESHOUSER EC, 1989, V264, P3740, J BIOL CHEM
SANDBERG M, 1987, V104, P1077, J CELL BIOL
SANDBERG M, 1989, V9, P82, MATRIX
SEKIGUCHI K, 1986, V25, P4936, BIOCHEMISTRY-US
VOGEL KG, 1984, V223, P587, BIOCHEM J
VOGEL KG, 1989, V49, P236, EUR J CELL BIOL
WU CH, 1991, V266, P2983, J BIOL CHEM
YAMAGUCHI Y, 1990, V346, P281, NATURE

13/9/42 (Item 42 from file: 73)
DIALOG(R)File 73:EMBASE

07097701 EMBASE No: 1997379565
Signal transduction targets involved in fibroproliferative vascular diseases

Molloy C.J.
Dr. C.J. Molloy, Cardiovascular Drug Discovery, Bristol-Myers Squibb, Pharmaceuticasl Research Institute, PO Box 4000, Princeton, NJ 08543 United States

Current Pharmaceutical Design (CURR. PHARM. DES.) (Netherlands) 1997, 3/6 (585-596)
CODEN: CPDEF ISSN: 1381-6128
DOCUMENT TYPE: Journal; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 155

Proliferation and directed migration of vascular cells are key components in fibroproliferative vascular diseases such as atherosclerosis and restenosis following percutaneous transluminal coronary angioplasty (PTCA). However, the precise cellular and molecular mechanisms involved in the

control of vascular cell proliferation or migration at the tissue level remain largely undefined. Molecules contributing to these processes are elaborated by distinct cell types and act in both autocrine and paracrine modes. They include two broad classes, polypeptide growth factors and vasoactive G-protein-coupled receptor (GPCR) agonists. Examples of the former, such as platelet-derived growth factor (PDGF), bind to and activate cell surface receptor tyrosine kinases, initiating intracellular biochemical signaling pathways associated with cell proliferation or migration. In contrast, recent evidence suggests that vasoactive GPCR agonists (e.g, angiotensin II (All), endothelin-l (ET-I), athrombin) elicit cell growth indirectly by inducing the production of autocrine or paracrine factors in vascular cells. This review summarizes recent studies on the cellular and molecular mechanisms involved in vascular smooth muscle cell growth control as well as provides information on possible therapeutic targets in this active area of vascular biological research.

DRUG DESCRIPTORS:
*growth factor--endogenous compound--ec; *guanine nucleotide binding protein--endogenous compound--ec
angiotensin--endogenous compound--ec; endothelin 1--endogenous compound--ec ; platelet derived growth factor--endogenous compound--ec; protein tyrosine kinase--endogenous compound--ec; thrombin--endogenous compound--ec
MEDICAL DESCRIPTORS:
*atherosclerosis--etiology--et; *restenosis--complication--co; *restenosis --etiology--et; *vascular disease--etiology--et
cell migration; cell proliferation; human; nonhuman; priority journal; review; signal transduction; transluminal coronary angioplasty
CAS REGISTRY NO.: 11128-99-7, 1407-47-2 (angiotensin); 80449-02-1 (**protein** tyrosine **kinase**); 9002-04-4 (thrombin)
SECTION HEADINGS:
002 Physiology
005 General Pathology and Pathological Anatomy
018 Cardiovascular Diseases and Cardiovascular Surgery
025 Hematology

13/9/46 (Item 46 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03015139 Genuine Article#: MW285 Number of References: 35
Title: IMMUNOHISTOCHEMICAL LOCALIZATION OF A NOVEL BETA(1) INTEGRIN IN NORMAL AND PATHOLOGICAL SQUAMOUS EPITHELIA
Author(s): CERRI A; FAVRE A; GIUNTA M; CORTE G; GROSSI CE; BERTI E
Corporate Source: UNIV GENOA,IST ANAT UMANA NORMALE,DEPT ANAT,VIATONI 14/I-16132 GENOA//ITALY/; UNIV GENOA,IST ANAT UMANA NORMALE,DEPT ANAT/I-16132 GENOA//ITALY/; UNIV MILAN,IRCCS,DEPT DERMATOL/MILAN//ITALY/; NATL INST CANC RES/GENOA//ITALY/
Journal: JOURNAL OF INVESTIGATIVE DERMATOLOGY, 1994, V102, N2 (FEB), P 247-252
ISSN: 0022-202X
Language: ENGLISH Document Type: ARTICLE
Geographic Location: ITALY
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences; CC CLIN--Current Contents, Clinical Medicine
Journal Subject Category: DERMATOLOGY & VENEREAL DISEASES
Abstract: The 10.1.2 MoAb reacts with a novel alpha chain that associates with the beta(1) integrin chain and is widely distributed among epithelial and endothelial cells of human adult and fetal tissues. In the epidermis and in other squamous epithelia, alpha(10.1.2) chains are expressed exclusively in the basal cell layer. Here we describe the immunohistochemical localization of alpha(10.1.2) in human epidermis, in other squamous epithelia, as well as in cultured keratinocytes. alpha(10.1.2) chain localization has also been investigated in a variety of non-neoplastic and neoplastic lesions of the skin, the uterine cervix, and the lung. We show that alpha(10.1.2) chains retain their basal keratinocyte localization in hyperplastic skin diseases and

in benign tumors of the epidermis and that they are strongly expressed in basal cell carcinomas. In contrast, alpha(10.1.2) expression is decreased in keratinocytes that differentiate in vitro and is lost in epidermal dysplastic conditions, in the invading front of squamous cell carcinomas of the epidermis, in microinvasive cervical cancers, and in well-differentiated squamous lung tumors. These findings indicate that alpha(10.1.2) beta(1) integrin is downregulated during keratinocyte differentiation in vitro and in vivo. Moreover, lack of alpha(10.1.2) expression in basal cells of squamous epithelia is associated with early dysplastic changes and with the acquisition of invasive capacity.

Descriptors--Author Keywords: ADHESION MOLECULES ; SKIN CANCER ; TUMOR INVASION ; METASTASIS

Identifiers--KeyWords Plus: CELL-ADHESION; HUMAN-SKIN; EXTRACELLULAR-MATRIX; EXPRESSION; RECEPTORS; KERATINOCYTES; LAMININ; BASAL; ALPHA-6-BETA-1; FIBRONECTIN

Research Fronts: 92-0486 001 (INTEGRIN EXPRESSION IN HUMAN-MELANOMA CELLS; TUMOR INVASION; TRANSFORMING GROWTH FACTOR-BETA-1; METASTATIC PHENOTYPE; EXTRACELLULAR-MATRIX INTERACTIONS)

92-0741 001 (ADHESION MOLECULES; REGULATION OF THE VLA INTEGRIN LIGAND INTERACTIONS; LFA-1 AVIDITY IN HUMAN B-CELLS; COSTIMULATORY SIGNAL)

92-3377 001 (BETA-1 INTEGRIN RECEPTORS; ADHESION MOLECULES; CELL-SURFACE EXPRESSION)

92-6678 001 (REGULATION OF THE VLA INTEGRIN LIGAND INTERACTIONS; FOCAL ADHESION-ASSOCIATED **PROTEIN TYROSINE KINASE** ; DIFFERENTIAL EXPRESSION; **SIGNAL TRANSDUCTION**)

Cited References:

ALBELDA SM, 1990, V4, P2868, FASEB J
BOYCE ST, 1985, V9, P83, J TISSUE CULTURE MET
CERRI A, 1992, V2, P279, EUR J DERMATOL
CORDELL JL, 1984, V32, P219, J HISTOCHEM CYTOCHEM
CROSSIN KL, 1990, V189, P172, ANN NY ACAD SCI
DELUCA M, 1990, V87, P6888, P NATL ACAD SCI USA
EDELMAN GM, 1991, V60, P155, ANN REV BIOCH
FAVRE A, 1992, V40, P1291, J HISTOCHEM CYTOCHEM
GIUNTA M, 1991, V173, P1537, J EXP MED
GROSSI CE, 1992, V4, P35, LEUKEMIA S4
HEMLER ME, 1990, V8, P365, ANNU REV IMMUNOL
HERTLE MD, 1991, V112, P193, DEVELOPMENT
HYNES RO, 1992, V69, P11, CELL
KELLNER I, 1991, V125, P211, BRIT J DERMATOL
MEDEIROS LJ, 1989, V63, P255, CANCER
MUELLER SC, 1991, V99, P213, J CELL SCI
PELTONEN J, 1989, V84, P1916, J CLIN INVEST
PLANTEFABER LC, 1989, V56, P281, CELL
RALFKIAER E, 1991, V124, P527, BRIT J DERMATOL
ROOSSIEN FF, 1989, V108, P1979, J CELL BIOL
RUOSLAHTI E, 1991, V87, P1, J CLIN INVEST
RUOSLATHI E, 1989, V1, P119, CANCER CELL
RUSCIANO D, 1992, V14, P185, BIOESSAYS
RYYNANEN J, 1991, V97, P562, J INVEST DERMATOL
SCHWARTZ MA, 1992, V2, P304, TRENDS CELL BIOL
SHIMIZU Y, 1991, V5, P2292, FASEB J
SOLLBERG S, 1992, V98, P864, J INVEST DERMATOL
SONNENBERG A, 1990, V110, P2145, J CELL BIOL
SONNENBERG A, 1991, V113, P907, J CELL BIOL
SONNENBERG A, 1990, V96, P207, J CELL SCI
SONNENBERG A, 1990, V96, P207, J CELL SCI
SPRINGER TA, 1990, V346, P425, NATURE
STAUDER R, 1989, V77, P234, CLIN EXP IMMUNOL
VANRIET I, 1991, V79, P421, BRIT J HAEMATOL
ZAMBRUNO G, 1991, V124, P135, BRIT J DERMATOL

13/9/8 (Item 8 from file: 155)

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  File 135:NewsRx Weekly Reports 1995-2005/Jan W4
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*File 135: New newsletters are now added. See Help News135 for the
complete list of newsletters.
  File 144:Pascal 1973-2005/Jan W4
        (c) 2005 INIST/CNRS
*File 144: Price change effective Jan 1, 2005. Enter HELP
RATES 144 for details.
  File 149:TGG Health&Wellness DB(SM) 1976-2005/Jan W4
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        (c) format only 2005 The Dialog Corporation
*File 156: Updating of ToxFile has resumed, with
UD=20041205.
  File 159:Cancerlit 1975-2002/Oct
        (c) format only 2002 Dialog Corporation
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Mechanisms of Disease: The Thyrotropin Receptor in Thyroid Diseases (Review Article)

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TEXT

The growth and function of the thyroid are controlled by thyrotropin (Ref. 1) through the activation of its receptor, which belongs to the large family of G protein-coupled receptors. Despite the extreme diversity of their ligands, all receptors from this family have a common molecular architecture: seven transmembrane segments, three extracellular loops, three intracellular loops, an extracellular amino terminal, and an intracytoplasmic carboxy terminal (Fig. 1). The glycoprotein hormone receptors constitute a subfamily that is characterized mainly by a particularly long amino-terminal extracellular domain that confers binding specificity. (Ref. 1,21)|*Figure 1.-The Thyrotropin Receptor.The location of constitutively activating mutations (Ref. 1-16) and inactivating mutations (Ref. 15,17,18) of the thyrotropin-receptor gene is shown, as is the location of somatic mutations found in thyroid carcinomas. (Ref. 10,19,20) At some locations, several different amino acid substitutions have been described. All gain-of-function mutations are in exon 10 except Ser281Asn/Thr, which is in exon 9. Gain-of-function mutations are denoted by circles in the case of hyperfunctioning thyroid adenomas, squares in the case of familial autosomal dominant hyperthyroidism, diamonds in the case of sporadic congenital hyperthyroidism, and octagons in the case of thyroid carcinomas. Loss-of-function mutations are denoted by triangles. Letters indicate the amino acid in the wild-type receptor. The asterisk and double asterisk indicate deletions resulting in a gain of function in hyperfunctioning thyroid adenomas *.**FIGURE OMITTED**

The thyrotropin receptor is encoded by 10 exons spread over 58 kilobases on chromosome 14. The large extracellular domain is encoded by the first nine exons, and the transmembrane segments and the carboxy terminal are encoded by exon 10. A number of splice variants of the receptor have been described, but their pathophysiologic importance is not known. (Ref. 1,21,22)

The thyrotropin receptor is preferentially coupled to the (alpha) subunit of the stimulatory guanine-nucleotide-binding protein (G(sub s)(alpha)) that activates adenylate cyclase and increases the accumulation of cyclic AMP (cAMP). At higher thyrotropin concentrations, the receptor also couples to the q subunit of guanine-nucleotide-binding protein alpha, resulting in the activation of phospholipase C, and there is recent evidence that the receptor may be coupled to members of other G protein families. (Ref. 23) In addition, insulin-like growth factor I, epidermal growth factor, transforming growth factor (beta), platelet-derived growth factor, fibroblast growth factor, and cytokines, mainly acting by means of the protein tyrosine kinase signal-transduction pathway, stimulate the growth and dedifferentiation of thyroid epithelial cells. (Ref. 1)

Both the growth and the function of the thyroid are stimulated by cAMP. (Ref. 1,24) This second messenger indirectly regulates the expression

of the thyroglobulin and thyroid peroxidase genes, whose promoters contain binding sites for the transcription factors TTF1, TTF2, and PAX8. (Ref. 25) As a consequence, continued stimulation of the cAMP pathway causes hyperthyroidism and thyroid hyperplasia (Fig. 2). The best examples of this are provided by Graves' disease, in which autoantibodies mimic the action of thyrotropin, (Ref. 26) and transgenic mice whose thyroids express the A2 adenosine receptor. (Ref. 27)|*Figure 2.-Consequences of the Activation of the Thyrotropin Receptor.The thyrotropin receptor is coupled mainly to the cAMP pathway by means of the (alpha) subunit of the stimulatory guanine-nucleotide-binding protein. The cAMP pathway regulates the production of thyroid hormone and the proliferation of thyroid epithelial cells and thereby mediates hyperthyroidism as well as the formation of thyroid adenomas. Both hormone production and the growth of thyroid epithelial cells are either stimulated (up arrow) or inhibited (down arrow) by local factors, such as insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), and transforming growth factor (beta) (TGF-(beta)) *.**FIGURE OMITTED**

Therefore, if a somatic mutation in a single thyroid epithelial cell caused chronic stimulation of the cAMP pathway, that cell would acquire a growth advantage and clonal expansion would result, leading to the formation of an autonomously functioning thyroid adenoma and ultimately to hyperthyroidism. Conversely, inhibition of this cascade by autoantibodies that block the thyrotropin receptor or by a defective thyrotropin receptor would result in hypothyroidism. In recent years alterations of the thyrotropin receptor and the regulatory pathways it controls have been identified in both hyperthyroidism and hypothyroidiAutonomously Functioning Thyroid Adenomas

In areas where there is sufficient iodine, hyperthyroidism due to Graves' disease is 50 times as prevalent as hyperthyroidism caused by an autonomously functioning thyroid adenoma. (Ref. 28) In contrast, in iodine-deficient areas, the causes of hyperthyroidism are different. For example, in East Jutland (Denmark) hyperthyroidism was caused by Graves' disease in 39 percent of patients, by autonomously functioning thyroid adenomas in 10 percent, and by toxic multinodular goiters in 48 percent. (Ref. 29) Therefore, iodine deficiency appears to promote the development of autonomously functioning thyroid adenomas.

These adenomas synthesize and secrete thyroid hormones autonomously, thereby suppressing thyrotropin secretion, so that the extranodular tissue becomes quiescent. On radionuclide imaging of the thyroid the adenomas are hyperfunctioning (``hot''), as compared with the paranodular thyroid tissue, which is deprived of thyrotropin stimulation. Depending on the iodine intake, growth potential, and other variables, it may take months to a decade or longer for an adenoma to grow large enough to cause hyperthyroidism. (Ref. 30)

The coexistence of autonomous and quiescent tissue in the thyroid gland suggests an inherent defect as the cause of autonomously functioning adenomas. This assumption is supported by the persistence of hyperactivity of adenoma cells in cell culture and after grafting into nude mice. (Ref. 31) Moreover, the occurrence of hyperthyroidism together with thyrotropin-independent growth of the adenoma suggests chronic activation of the cAMP cascade (Fig. 2). Somatic mutations in a gene of the cAMP regulatory cascade leading to constitutive activation (e.g., activation in the absence of stimulatory ligand) of this cascade were first detected in G(sub s)(alpha) in pituitary somatotroph adenomas. (Ref. 32) Subsequently, G(sub s)(alpha) mutations were identified in 12 to 38 percent of autonomously functioning thyroid adenomas. (Ref. 33)

G protein-coupled receptors have extensive homology in the transmembrane regions involved in receptor activation, as demonstrated by studies of site-directed mutagenesis with another G protein-coupled receptor, the (alpha)1b-adrenergic receptor. (Ref. 34) Therefore, it seemed logical to screen the same region of the thyrotropin-receptor gene for somatic mutations in autonomously functioning thyroid adenomas. The first mutations were identified in the third intracellular loop of the receptor (Ref. 2) (Fig. 1), but mutations in other regions were subsequently detected. (Ref. 2-4) Some of the affected patients were clinically euthyroid, but most had hyperthyroidism. To date, 28 substitutions in 21 amino acid residues conferring constitutive activity to the thyrotropin

receptor have been identified (including those found in patients with familial or sporadic congenital nonautoimmune hyperthyroidism) (Fig. 1). When the function of these mutants was assessed by transfection of recombinant constructs into COS cells, they all increased thyrotropin-independent cAMP production over that induced by the wild-type receptor. (Ref. 1,2-12) Constitutively activating mutations were also identified in the genes for the luteinizing hormone receptor in boys with precocious puberty, (Ref. 35) the parathyroid hormone receptor in patients with Jansen's metaphyseal chondrodysplasia, (Ref. 36) and the follicle-stimulating hormone receptor in a man with sustained spermatogenesis who had undergone hypophysectomy. (Ref. 37) Constitutively activating mutations in G protein-coupled receptors are therefore emerging as a new pathophysiologic entity in endocrinology.

The frequency of mutations in the thyrotropin-receptor gene in autonomously functioning thyroid adenomas varies with the sensitivity of the detection method used (direct sequencing being more sensitive than analysis involving single-strand conformation polymorphisms), the extent of the region screened for mutations, the quality of the tissue examined (mutations are more difficult to find in highly fragmented DNA extracted from paraffin-embedded tissue than in frozen tissue), and the type of tissue sampling (surgical specimens vs. those obtained by fine-needle aspiration biopsy). In addition, other factors such as the genetic background and the iodine intake might influence the incidence of thyrotropin-receptor gene mutations in the adenomas. In studies reporting low frequencies (0 or 8 percent) of mutations in the thyrotropin-receptor gene, only parts of exon 10 were studied, (Ref. 10,38) whereas in studies reporting high frequencies (48 to 80 percent) nearly all of exon 10 was sequenced. (Ref. 4,12,13,39) In a study in which all of exon 10 was sequenced in 44 adenomas, the incidence of mutations was 20 percent. (Ref. 40) However, in the same study G(sub s)(alpha) mutations were found in 24 percent of the adenomas. In two other studies in which the incidence of mutations in exon 10 of the thyrotropin-receptor gene was high (70 and 48 percent), the incidence of G(sub s)(alpha) mutations was low (0 and 4 percent, respectively). (Ref. 12,13) Mutations leading to constitutive activation of the cAMP cascade appear to be the cause of a substantial proportion (48 to 70 percent) of autonomously functioning thyroid adenomas.

Mutations in the thyrotropin-receptor gene were detected in 5 of 12 hyperfunctioning nodules in six patients with toxic multinodular goiters, each of whom had 2 hyperfunctioning nodules. (Ref. 8) Furthermore, cAMP-independent pathways have been implicated in goitrogenesis, (Ref. 24) suggesting the action of different, partly overlapping pathophysiologic mechanisms in the heterogeneous disorder of toxic multinodular goiter.

Differentiated Thyroid Cancer

The demonstration of the oncogenic potential of the cAMP pathway in benign hyperfunctioning thyroid adenomas prompted investigation of the role of this pathway in the pathogenesis of differentiated thyroid carcinomas. Mutations in G(sub s)(alpha) were found in 7 of 61 carcinomas in two studies. (Ref. 41,42) In two other studies, constitutively activating mutations in the thyrotropin-receptor gene were detected in 5 of 44 carcinomas (Ref. 19,20); no G(sub s)(alpha) mutations were found in any of the tumors. All the mutations in the thyrotropin-receptor gene in these tumors had previously been identified in autonomously functioning adenomas (Fig. 1). Moreover, in one patient a thyroid carcinoma with a somatic mutation resulted in the production of sufficient thyroid hormone to cause hyperthyroidism. (Ref. 20)

Constitutive activation of the cAMP pathway in the thyroid leads to progressive growth of thyrocytes, while usually maintaining cell differentiation. (Ref. 24) However, the thyroid carcinomas with G(sub s)(alpha) mutations did not take up iodine, (Ref. 19) suggesting a second, dedifferentiating genetic alteration. In agreement with a multistep model of thyroid carcinogenesis, the presence of a G(sub s)(alpha) or a thyrotropin-receptor gene mutation together with an activated ras gene has been demonstrated in differentiated carcinoma. (Ref. 19) It is therefore likely that both the ras and G(sub s)(alpha) or thyrotropin-receptor gene mutations act synergistically to cause the tumor phenotype in some differentiated thyroid carcinomas. However, thyrotropin-receptor gene or G(sub s)(alpha) mutations in differentiated thyroid carcinomas are rare,

and chronic activation of the cAMP pathway probably does not increase the likelihood of further genetic damage, as suggested by the rarity of malignant transformation in autonomously functioning thyroid adenomas, Graves' disease, and the benign toxic thyroid hyperplasia in transgenic mice with chronic stimulation of the cAMP pathway by ectopic expression of the adenosine A2 receptor (Ref. 27) or G(sub s)(alpha) mutations (Ref. 43) in the thyroid gland.

Germ-line Mutations in the Thyrotropin Receptor in Autosomal Dominant Nonautoimmune Hyperthyroidism

Familial clustering of hyperthyroidism due to Graves' disease is a well-known phenomenon. Autosomal dominant inheritance of nonautoimmune hyperthyroidism was described 15 years ago. (Ref. 44) Once somatic mutations in the thyrotropin-receptor gene and G(sub s)(alpha) had been demonstrated in autonomously functioning thyroid adenomas, families with nonautoimmune hypothyroidism were reevaluated for germ-line mutations in the thyrotropin-receptor gene.

Sequencing of the thyrotropin-receptor gene in two families with hereditary nonautoimmune hyperthyroidism led to the identification of constitutively activating heterozygous germ-line mutations. (Ref. 45) The functional in vitro characteristics of these two mutations were similar to those already described for autonomously functioning thyroid adenomas, (Ref. 1) and thus explain the development of thyroid hyperplasia and hyperthyroidism in affected patients.

Six other families with different mutations in the thyrotropin-receptor germ line have subsequently been identified. (Ref. 7,9,11,46) These patients do not have the clinical manifestations of hyperthyroidism due to Graves' disease, such as thyroid-associated ophthalmopathy, pretibial myxedema, or lymphocytic infiltration of the thyroid, nor do they have any thyroid antibodies. The thyroid gland is enlarged in most patients. Hyperthyroidism can occur at any time from the neonatal period to adulthood. This variability in the age at onset is probably the result of other genetic components and environmental factors such as iodine intake and dietary goitrogens. Patients require ablative treatment (surgery or radioiodine), because recurrent hyperthyroidism after subtotal thyroidectomy, mandating a second thyroidectomy or radioiodine treatment, has been reported in many families.

Germ-line mutations can be inherited, or they can arise spontaneously. In four infants with sporadic congenital hyperthyroidism, germ-line mutations in the thyrotropin-receptor gene were identified. (Ref. 9,14,17,47) All four infants had severe, persistent hyperthyroidism without thyroid antibodies. In all cases, both parents were euthyroid and none had germ-line mutations in the thyrotropin-receptor gene or Graves' disease. These patients can therefore be classified as having sporadic congenital nonautoimmune autosomal dominant hyperthyroidism. Two of the four patients were treated by thyroidectomy because of persistent hyperthyroidism during treatment with an antithyroid drug and rapidly enlarging goiters. (Ref. 17,47)

As shown in Figure 1, a number of mutations in the thyrotropin-receptor gene have been found both as somatic mutations in patients with autonomously functioning thyroid adenomas and as germ-line mutations in patients with autosomal dominant nonautoimmune hyperthyroidism. This is convincing evidence of a common pathophysiologic mechanism. Moreover, sporadic nonautoimmune hyperthyroidism can eventually become an inherited disease. Very early onset of hyperthyroidism has been reported in two women whose children had congenital nonautoimmune hyperthyroidism. (Ref. 12,46)

The question of whether the various mutations in the thyrotropin-receptor gene cause different activities of the cAMP pathway was addressed by preliminary parallel in vitro studies of 11 mutations. (Ref. 1) The basal activities of the mutations varied widely. Most of the mutations activated only the cAMP cascade, but five (I486M, A623I, I568T, T632I, and I486F) also activated the phospholipase C-dependent cascade. However, the in vitro activities of the somatic and germ-line mutations were similar, and there is no apparent phenotypic difference between patients with different mutations in hot nodules or families with germ-line mutations in well-conserved as opposed to nonconserved amino acid residues.

Nonetheless, some preliminary diagnostic and therapeutic conclusions

can be drawn on the basis of the clinical characteristics of the patients with germ-line mutations in the thyrotropin-receptor gene. In families in which multiple members have nonautoimmune hyperthyroidism and in persons with sporadic congenital hyperthyroidism and no evidence of an autoimmune cause, a search for mutations in the thyrotropin-receptor gene is indicated. Only the identification of a mutation will lead to a definitive diagnosis. Patients with germ-line mutations should be treated early by removing as much thyroid tissue as possible to control the hyperthyroidism permanently and avoid relapses.

Thyrotropin Resistance

Several reports of patients with nonautoimmune congenital hypothyroidism have suggested that there is a syndrome of resistance of the thyroid to thyrotropin. (Ref. 48-50) All the affected patients have had normal-sized thyroid glands but high serum concentrations of biologically active thyrotropin. The absence of thyroglobulin in the thyroid gland in one patient and a decreased uptake of radioiodine by the thyroid in another patient are consistent with an impaired cAMP-signaling system as the cause of reduced synthesis of thyroid-specific proteins and impaired proliferation of thyroid epithelial cells (Fig. 2). Additional evidence of a putative abnormality in the thyrotropin-receptor-adenylate cyclase system was provided by the demonstration of thyrotropin unresponsiveness both in vivo and in vitro. (Ref. 49,50)

Thyrotropin resistance can be defined as reduced responsiveness or nonresponsiveness of the thyroid gland to biologically active thyrotropin. It can be caused by defects in the receptor itself or in elements involved in the transduction of signals from the receptor into the cell. Contrary to the dominant gain-of-function mutations in the thyrotropin-receptor gene in autonomously functioning thyroid adenomas, which are heterozygous (only one mutated allele), loss-of-function mutations probably require mutations in both alleles in order to be expressed, unless the product of the mutated allele interferes with the expression of the wild-type allele. In keeping with this hypothesis, the first loss-of-function point mutation in the thyrotropin-receptor gene, which was identified in hypothyroid (hyt/hyt) mice, affected both alleles. (Ref. 51)

The molecular basis of thyrotropin resistance in humans was recently identified in three siblings who were euthyroid and had normal serum thyroid hormone concentrations but high serum thyrotropin concentrations. All three patients were compound heterozygotes, each having a point mutation in each thyrotropin-receptor allele; the heterozygous parents were asymptomatic. Whereas the paternal allele (Ile167Ala) had completely lost the capacity to be activated by thyrotropin, the maternal allele (Pro162Ala) had only reduced sensitivity to the hormone. (Ref. 52) Subsequently, six more families with thyrotropin resistance have been identified. (Ref. 15,18,53,54) The majority of the probands were identified on the basis of elevated blood thyrotropin concentrations at the time of neonatal screening. However, in contrast to patients with congenital hypothyroidism, nearly all the patients had normal serum thyroid hormone concentrations. (Ref. 15) Their thyroid glands were normal-sized, a hypoplastic thyroid gland being reported in only one patient. (Ref. 18) However, in three patients with congenital hypothyroidism and no thyroid enlargement who were unresponsive to thyrotropin, no mutation in the thyrotropin-receptor gene was found. (Ref. 55) Therefore, defects in other genes or flanking regions of the thyrotropin receptor might also cause thyrotropin resistance.

The Thyrotropin Receptor and Autoimmunity

In addition to its role in nonautoimmune hyperthyroidism and hypothyroidism, the thyrotropin receptor is also an autoantigen. However, in this case the receptor seems to be a bystander, because mutations in the receptor originally thought to be implicated in patients with Graves' disease have been found in their normal relatives and in unrelated **normal** subjects. (Ref. 1,22)

The thyrotropin receptor is the target of both thyroid-stimulating antibodies (as in Graves' hyperthyroidism) and thyroid-blocking antibodies (as in chronic autoimmune thyroiditis). The majority of thyroid-stimulating antibodies and thyroid-blocking antibodies are also immunoglobulins that inhibit the binding of thyrotropin. (Ref. 56) Both types of antibodies may coexist in the same patient. (Ref. 26)

Unlike other antibodies (e.g., antithyroperoxidase and antithyroglobulin antibodies), (Ref. 57) thyrotropin-receptor antibodies are directly involved in the pathogenesis of Graves' disease, as demonstrated by the occurrence of transient hyperthyroidism in neonates whose mothers have thyroid-stimulating antibodies. (Ref. 26) However, serum obtained from a minority of untreated patients with hyperthyroidism due to Graves' disease contains no **detectable** thyrotropin-receptor antibodies. This may be due to the insensitivity of the current **assays** or because these patients do not have Graves' disease.

In an attempt to understand why thyrotropin-receptor antibodies may be thyrotropin agonists and with the aim of developing improved **assays** for these antibodies, considerable effort has been spent to define the regions of the thyrotropin receptor involved in the binding and bioactivity of thyrotropin, thyrotropin-receptor-stimulating antibodies, and thyrotropin-receptor-binding antibodies. The main findings are that both thyrotropin and thyrotropin-receptor antibodies bind to numerous discontinuous residues throughout the extracellular region of the receptor (i.e., their binding sites and their bioactivity depend on the recognition of the three-dimensional structure of the thyrotropin receptor). (Ref. 58,59) The finding that there is binding to different regions of the thyrotropin receptor provides some explanation for the existence of antibodies having agonist or antagonist activities, but further progress in this area awaits the elucidation of the three-dimensional structure of the receptor and of the mechanism of receptor activation.

Conclusions

The thyrotropin receptor is involved in a wide range of sporadic and hereditary or genetically **determined** changes in thyroid function, most likely because small changes in amino acid composition readily alter its configuration, resulting in increased intrinsic activity or decreased responsiveness to thyrotropin. In Graves' disease the thyrotropin receptor is the target of thyroid-stimulating and thyroid-blocking antibodies.

This article is dedicated to the memory of Olaf Heine, M.D

CITED REFERENCES

1. Van Sande J, Parma J, Tonacchera M, Swillens S, Dumont J, Vassart G. Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab 1995;80:2577-85.
2. Parma J, Duprez L, Van Sande J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature 1993;365:649-51.
3. Paschke R, Tonacchera M, Van Sande J, Parma J, Vassart G. Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. J Clin Endocrinol Metab 1994;79:1785-9.
4. Porcellini A, Ciullo I, Laviola L, Amabile G, Fenzi G, Avvedimento VE. Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas: rapid identification by fine needle aspiration biopsy. J Clin Endocrinol Metab 1994;79:657-61.
5. Kosugi S, Shenker A, Mori T. Constitutive activation of cyclic AMP but not phosphatidylinositol signaling caused by four mutations in the 6th transmembrane helix of the human thyrotropin receptor. FEBS Lett 1994;356:291-4.
6. Tonacchera M, Van Sande J, Cetani F, et al. Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. J Clin Endocrinol Metab 1996;81:547-54.
7. Fuhrer D, Willgerodt H, Scherbaum WA, Paschke R. Identification of a new germline mutation in the thyrotropin receptor gene as a cause of familial nonautoimmune hyperthyroidism. J Clin Endocrinol Metab (in press).
8. Holzapfel HP, Fuhrer D, Wonerow P, Weinland G, Scherbaum WA, Paschke R. Identification of constitutively activating somatic thyrotropin receptor mutations in a subset of toxic multinodular goitres. J Clin Endocrinol Metab (in press).
9. De Roux N, Polak M, Couet J, et al. A neomutation of the

thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism. J Clin Endocrinol Metab 1996;81:2023-6.
10. Russo D, Arturi F, Wicker R, et al. Genetic alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab 1995;80:1347-51.
11. Kohler B, Biebermann H, Krohn HP, Dralle D, Finke R, Gruters A. A novel germline mutation in the thyrotropin receptor gene causing nonautoimmune congenital hyperthyroidism. In: Volume I (June 12-13) of program & abstracts of the 10th International Congress of Endocrinology (ICE '96), San Francisco, June 12-15, 1996. Bethesda, Md.: Endocrine Society Press, 1996:641. abstract.
12. Fuhrer D, Holzapfel HP, Wonerow P, Scherbaum WA, Paschke R. Screening for mutations in the thyrotropin receptor and gsp genes in toxic thyroid nodules. J Clin Endocrinol (in press).
13. Parma J, Duprez L, Van Sande J, et al. Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs (alpha) genes as a cause of toxic thyroid adenomas. J Clin Endocrinol Metab 1997;82:2695-701.
14. Esapa C, Betts P, Kendall-Taylor P, Harris PE. A novel TSH receptor mutation in an infant with thyrotoxicosis. J Endocrinol Invest 1996;19:Suppl 6:71. abstract.
15. Clifton-Bligh RJ, Gregory JW, Ludgate R, et al. Two novel mutations in the thyrotropin (TSH) receptor gene in a child with resistance to TSH. J Clin Endocrinol Metab 1996;82:1094-100.
16. Russo D, Belfiore A, Tumino S, et al. A thyroid follicular cancer presenting as a hot nodule and hyperfunctioning because of an activating mutation of the TSH receptor. J Endocrinol Invest 1996;19:Suppl 6:1. abstract.
17. Kopp P, van Sande J, Parma J, et al. Congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. N Engl J Med 1995;332:150-4.
18. Biebermann H, Krude H, Thiede C, Kotulla D, Gruters A. Sporadic congenital hypothyroidism due to compound heterozygosity for two mutations of the coding sequence of the thyrotropin receptor gene. In: Volume I (June 12-13) of program & abstracts of the 10th International Congress of Endocrinology (ICE '96), San Francisco, June 12-15, 1996. Bethesda, Md.: Endocrine Society Press, 1996:643. abstract.
19. Russo D, Arturi F, Schlumberger M, et al. Activating mutations of the TSH receptor in differentiated thyroid carcinomas. Oncogene 1995;11:1907-11.
20. Spambalg D, Sharifi N, Elisi R, Gross JL, Medeiros-Neto G, Fagin JA. Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. J Clin Endocrinol Metab 1996;81:3898-901.
21. Paschke R, Van Sande J, Parma J, Vassart G. The TSH receptor and thyroid disease. Baillieres Clin Endocrinol Metab 1996;10:9-27.
22. Paschke R, Vassart G, Ludgate M. Current evidence for and against the TSH receptor being the common antigen in Graves' disease and thyroid associated ophthalmopathy. Clin Endocrinol (Oxf) 1995;42:565-9.
23. Laugwitz K-L, Allgeier A, Offermanns S, et al. The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. Proc Natl Acad Sci U S A 1996;93:116-20.
24. Dumont JE, Maenhaut C, Pirson I, Baptist M, Roger PP. Growth factors **controlling** the thyroid gland. Baillieres Clin Endocrinol Metab 1991;5:727-54.
25. Damante G, DiLauro R. Thyroid-specific gene expression. Biochim Biophys Acta 1994;1218:255-66.
26. McKenzie JM, Zakarija M. The clinical use of thyrotropin receptor antibody **measurements** . J Clin Endocrinol Metab 1989;69:1093-6.
27. Ledent C, Dumont JE, Vassart G, Parmentier M. Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J 1992;11:537-42.
28. Hamburger JI. Evolution of toxicity in solitary nontoxic autonomously functioning thyroid nodules. J Clin Endocrinol Metab 1980;50:1089-93.
29. Laurberg P, Pedersen KM, Vestergaard H, Sigurdsson G. High incidence of multinodular toxic goitre in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a

high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland. J Intern Med 1991;229:415-20.
30. Sandrock D, Olbricht T, Emrich D, Benker G, Reinwein D. Long-term follow- up in patients with autonomous thyroid adenoma. Acta Endocrinol (Copenh) 1993;128:51-5.
31. Usadel KH, Paschke R, Teuber J, Schwedes U. Hetero-transplantation of autoimmune human thyroid to nude mice as a tool for in vivo autoimmune research. In: Pinchera A, Ingbar SH, McKenzie JM, Fenzi GF, eds. Thyroid autoimmunity. New York: Plenum Press, 1987:207-10.
32. Lyons J, Landis CA, Harsh G, et al. Two G protein oncogenes in human endocrine tumors. Science 1990;249:655-9.
33. Spiegel AM, Weinstein LS, Shenker A. Abnormalities in G protein-coupled **signal transduction** pathways in human disease. J Clin Invest 1993;92:1119-25.
34. Kjelsberg MA, Cotecchia S, Ostrowski J, Caron MG, Lefkowitz RJ. Constitutive activation of the (alpha) 1B-adrenergic receptor by all amino acid substitutions at a single site: evidence for a region which constrains receptor activation. J Biol Chem 1992;267:1430-3.
35. Shenker A, Laue L, Kosugi S, Merendino JJ Jr, Minegishi T, Cutler GB Jr. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature 1993;365:652-4.
36. Schipani E, Langman CB, Parfitt AM, et al. Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia. N Engl J Med 1996;335:708-14.
37. Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. J Clin Endocrinol Metab 1996;81:1367-70.
38. Takeshita A, Nagayama Y, Yokoyama N, et al. Rarity of oncogenic mutations in the thyrotropin receptor of autonomously functioning thyroid nodules in Japan. J Clin Endocrinol Metab 1995;80:2607-11.
39. Parma J, Van Sande J, Swillens S, Tonacchera M, Dumont JE, Vassart G. Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3',5'-monophosphate and inositol phosphate-Ca2+ cascades. Mol Endocrinol 1995;9:725-33.
40. Russo D, Arturi F, Suarez HG, et al. Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab 1996;81:1548-51.
41. Suarez HG, du Villard JA, Caillou B, Schlumberger M, Parmentier C, Monier R. Gsp mutations in human thyroid tumours. Oncogene 1991;6:677-9.
42. Yoshimoto K, Iwahana H, Fukuda A, Sano T, Itakura M. Rare mutations of the Gs alpha subunit gene in human endocrine tumors: mutation **detection** by polymerase chain reaction-primer-introduced restriction analysis. Cancer 1993;72:1386-93.
43. Michiels FM, Caillou B, Talbot M, et al. Oncogenic potential of guanine nucleotide stimulatory factor alpha subunit in thyroid glands of transgenic mice. Proc Natl Acad Sci U S A 1994;91:10488-92.
44. Thomas JS, Leclere J, Hartemann P, et al. Familial hyperthyroidism without evidence of autoimmunity. Acta Endocrinol (Copenh) 1982;100:512-8.
45. Duprez L, Parma J, Van Sande J, et al. Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. Nat Genet 1994;7:396-401.
46. Schwab KO, Sohlemann P, Gerlich M, et al. Mutations of the TSH receptor as a cause of congenital hyperthyroidism. Exp Clin Endocrinol Diabetes 1996;104:Suppl 4:124-8.
47. Holzapfel H-P, Wonerow P, Petrykowski W, Henschen M, Scherbaum WA, Paschke R. Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. J Clin Endocrinol Metab (in press).
48. Takamatsu J, Nishikawa M, Horimoto M, Ohsawa N. Familial unresponsiveness to thyrotropin by autosomal recessive inheritance. J

Clin Endocrinol Metab 1993;77:1569-73.
49. Codaccioni JL, Carayon P, Michel-Bechet M, Foucault F, Lefort G, Pierron H. Congenital hypothyroidism associated with thyrotropin unresponsiveness and thyroid cell membrane alterations. J Clin Endocrinol Metab 1980;50:932-7.
50. Medeiros-Neto GA, Knobel M, Bronstein MD, Simonetti J, Filho FF, Mattar E. Impaired cyclic-AMP response to thyrotropin in congenital hypothyroidism with thyroglobulin deficiency. Acta Endocrinol (Copenh) 1979;92:62-72.
51. Stein SA, Oates EL, Hall CR, et al. Identification of a point mutation in the thyrotropin receptor of the hyt/hyt hypothyroid mouse. Mol Endocrinol 1994;8:129-38.
52. Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, Refetoff S. Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. N Engl J Med 1995;332:155-60.
53. Asteria C, Persani L, Romoli R, Beck-Peccoz P. Resistance to thyrotropin action resulting from inactivating mutation of thyrotropin receptor (TSH-R) gene. J Endocrinol Invest 1996;19:Suppl 6:26. abstract.
54. DeRoux N, Misrahi M, Brauner R, et al. Four families with loss of function mutations of the thyrotropin receptor. J Clin Endocrinol Metab 1996;81:4229-35.
55. Takeshita A, Nagayama Y, Yamashita S, et al. Sequence analysis of the thyrotropin (TSH) receptor gene in congenital primary hypothyroidism associated with TSH unresponsiveness. Thyroid 1994;4:255-9.
56. Rees Smith B, McLachlan SM, Furmaniak J. Autoantibodies to the thyrotropin receptor. Endocr Rev 1988;9:106-21.
57. Ludgate M, Vassart G. The molecular genetics of three thyroid autoantigens: thyroglobulin, thyroid peroxidase and the thyrotropin receptor. Autoimmunity 1990;7:201-11.
58. Kohn LD, Shimura H, Shimura Y, et al. The thyrotropin receptor. Vitam Horm 1995;50:287-384.
59. McLachlan S, Rapoport B. Recombinant thyroid autoantigens: the keys to the pathogenesis of autoimmune thyroid disease. J Intern Med 1993;234:347-59.

13/9/14 (Item 14 from file: 73)

05580147 EMBASE No: 1993348247

Hyperactivation of signal transduction systems in Alzheimer's disease

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DOCUMENT TYPE: Journal; Conference Paper
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

A compromise or deregulation in signal transduction cascades could adversely affect cellular functions and possibly contribute to cell death. In recent years, it has become increasingly apparent that pronounced activation of neuronal signal transduction systems is a characteristic of AD brain. There is evidence that signal transduction systems play a role in the formation or development of these pathological features of AD. Aberrant activity and localization of components of signaling mechanisms (growth factors, their receptors, protein kinases, phosphoprotein phosphatases, and phosphoproteins) are closely associated with the intracellular accumulation of PHF, the extracellular deposition of amyloid, and the formation of neuritic plaques in AD brain. In particular, immunohistochemical studies reveal increased levels of neuronal staining for APP, possibly an important growth factor in AD, both in frontal cortex and hippocampus. Anti-APP immunostaining is also associated with the neuritic component of plaques. Additionally, PKC(betaII) immunostaining is increased in the neuronal cell

body and neuropil of AD samples, particularly in association with plaques, suggesting a postsynaptic involvement of this enzyme. On the other hand, PKC(betaI) immunostaining is associated with axonal staining particularly in the sprouting neurites of plaques. Sprouting neuritic components of plaques are immunopositive with other growth-associated proteins, such as GAP43, MARCKS, and spectrin. Immunoreactivity of other members of signal transduction systems such as Fos and stathmin are all increased in AD hippocampal neurons. On the other hand, several protein kinases and phosphoproteins were immunolocalized to tangles. Thus, the hyperactivation and dysfunction of signal transduction systems could be involved in the pathogenesis of AD.

DRUG DESCRIPTORS:
growth factor receptor; amyloid protein--endogenous compound--ec; casein kinase ii--endogenous compound--ec; growth associated protein--endogenous compound--ec; growth factor--endogenous compound--ec; neuromodulin --endogenous compound--ec; phosphoprotein--endogenous compound--ec; phosphoprotein phosphatase--endogenous compound--ec; phosphotyrosine --endogenous compound--ec; protamine kinase--endogenous compound--ec; protein kinase c--endogenous compound--ec; protein tyrosine kinase --endogenous compound--ec; spectrin--endogenous compound--ec; stathmin --endogenous compound--ec; transcription factor--endogenous compound--ec
MEDICAL DESCRIPTORS:
*alzheimer disease--etiology--et; *signal transduction
apoptosis; cell death; cell function; cellular distribution; conference paper; controlled study; enzyme activity; frontal cortex; hippocampus; human; human tissue; immunohistochemistry; nerve conduction; nerve fiber; neurite; neuropil; perikaryon; priority journal; etiology
CAS REGISTRY NO.: 9025-75-6 (phosphoprotein phosphatase); 21820-51-9 (phosphotyrosine); 9068-21-7 (protamine kinase); 141436-78-4 (protein kinase c); 80449-02-1 (**protein** tyrosine kinase); 12634-43-4 (spectrin); 126880-56-6 (stathmin)
SECTION HEADINGS:
  008 Neurology and Nerosurgery
  029 Clinical and Experimental Biochemistry

13/9/17 (Item 17 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02882104 Genuine Article#: MN408 Number of References: 37
Title: IL-2-INDUCED EXPRESSION OF TTK, A SERINE, THREONINE, TYROSINE KINASE, CORRELATES WITH CELL-CYCLE PROGRESSION
Author(s): SCHMANDT R; HILL M; AMENDOLA A; MILLS GB; HOGG D
Corporate Source: TORONTO GEN HOSP,DIV ONCOL RES,CCRW 3-827,200 ELIZABETH ST/TORONTO M5G 2C4/ON/CANADA/; TORONTO GEN HOSP,DIV ONCOL RES,CCRW 3-827,200 ELIZABETH ST/TORONTO M5G 2C4/ON/CANADA/
Journal: JOURNAL OF IMMUNOLOGY, 1994, V152, N1 (JAN 1), P96-105
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Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: IMMUNOLOGY
Abstract: We have recently isolated the cDNA for a unique human 97-kDa kinase, TTK, by expression screening of a cDNA expression library using anti-phosphotyrosine antibodies. When expressed in Escherichia coli, TTK can phosphorylate serine, threonine, and tyrosine residues. Thus TTK appears to belong to a newly described family of kinases able to phosphorylate all three hydroxy amino acids. This family of multispecific kinases includes several other kinases involved in cell cycle progression. In support of a possible role in regulating cell cycle progression, TTK message is readily detected in rapidly proliferating tissues in vivo including testes, thymus, bone marrow, and many malignant tumors, but not in benign tissues with a low proliferative rate in vivo. To determine the effect of cell activation and cell cycle progression on TTK expression, we measured TTK mRNA and

protein levels as well as kinase activity in freshly isolated T cells or IL-2-expanded T cell blasts activated to proliferate by the addition of a variety of mitogens. TTK mRNA levels, protein levels, and kinase activity were greatly enhanced when either freshly isolated PBL or T cell blasts were activated by cross-linking the TCR complex by mitogenic lectins or by bypassing the TCR with phorbol esters and cation ionophores. Incubation with IL-2 increased TTK expression in PBL blasts, which proliferate in response to IL-2, but not in fresh PBL, which do not proliferate in response to IL-2. TTK expression was blocked by either cyclosporin A or FK520, which inhibit IL-2 production and could be recovered by the addition of exogenous IL-2. Furthermore, TTK expression was prevented by incubation of the cells with rapamycin, which blocks IL-2 signaling. Thus, TTK expression in T cells appears to be a consequence of IL2-induced cell proliferation. Agonist-induced TTK expression was a delayed event occurring 1 2 to 24 h after activation of PBL blasts and 48 to 72 h after activation of fresh PBL. TTK protein and mRNA expression increased in both fresh PBL and T cell blasts concurrently with passage of cells through S phase as indicated by [H-3]TdR incorporation and cell cycle analysis of propidium iodide-stained cells. TTK mRNA and protein levels reached a maximum as cells entered the G2 phase of the cell cycle. These results were confirmed by cell cycle blockade studies with aphidicolin and nocodazole wherein TTK protein levels are not detected in cells in G1 and are readily detectable in cells in the S and G2 phases of the cell cycle. Furthermore, changes in TTK mRNA levels in activated cells paralleled those of cyclin A, which is expressed in late S phase and in G2 and were markedly different from those of cyclin D2, which is expressed in G1. Taken together, the data suggest that TTK may play a role in IL-2-induced passage of T cells through the S and G2M phases of the cell cycle.

Identifiers--KeyWords Plus: PROTEIN-KINASE; CYCLOSPORINE-A; PHOSPHORYLATION; ACTIVATION; RECEPTOR; P34CDC2; IDENTIFICATION; PROLIFERATION; INHIBITOR; RAPAMYCIN

Research Fronts: 91-0893 002 (TYROSINE PHOSPHORYLATION; INVITRO ACTIVATION OF A MYELIN BASIC-PROTEIN MICROTUBULE-ASSOCIATED PROTEIN-2 KINASE; THREONINE RESIDUES)

91-0133 001 (RETINOBLASTOMA PROTEIN; TUMOR SUPPRESSOR GENES; P53 MUTATIONS; HUMAN PAPILLOMAVIRUS TYPE-16 E7)

91-0237 001 (GTPASE-ACTIVATING PROTEIN; KIT RECEPTOR TYROSINE **KINASE** ; SIGNAL TRANSDUCTION; TYPE-1 NEUROFIBROMATOSIS GENE; RAS SUPERFAMILY)

91-6704 001 (T-CELL ACTIVATION VIA THE T-CELL RECEPTOR; DIFFERENTIAL REGULATION; CD2 SURFACE EXPRESSION)

Cited References:

ANDERSON NG, 1990, V343, P651, NATURE
BENDAVID Y, 1991, V10, P317, EMBO J
BIERER BE, 1991, V23, P2850, TRANSPLANT P
BISCHOFF JR, 1990, V87, P4766, P NATL ACAD SCI USA
BRUGG B, 1991, V114, P735, J CELL BIOL
BUCHKOVICH K, 1989, V58, P1097, CELL
CANTRELL DA, 1984, V224, P1312, SCIENCE
DOUVILLE EMJ, 1992, V12, P2681, MOL CELL BIOL
FEATHERSTONE C, 1991, V349, P808, NATURE
FELIX MA, 1990, V346, P379, NATURE
HANKS SK, 1991, V200, P38, METHOD ENZYMOL
HOWELL BW, 1991, V11, P568, MOL CELL BIOL
HUBERMAN JA, 1981, V23, P264, CELL
HUNTER T, 1992, V70, P372, CELL
HUNTER T, 1991, V200, P1, METHOD ENZYMOL
ICELY PL, 1991, V266, P6073, J BIOL CHEM
KLAUSNER RD, 1991, V64, P875, CELL
KREK W, 1991, V10, P305, EMBO J
LALANDE M, 1990, V186, P332, EXP CELL RES
LUCAS JJ, 1992, V148, P1804, J IMMUNOL
MILLS GB, 1991, V19, P99, BIOCHEM SOC T
MILLS GB, 1992, V267, P6000, J BIOL CHEM
MILLS GB, 1986, V136, P1150, J IMMUNOL
MORENO S, 1989, V58, P361, CELL

MORRISON DK, 1989, V58, P649, CELL
PARKER LL, 1992, V89, P2917, P NATL ACAD SCI USA
PELECH SL, 1990, V68, P1297, BIOCHEM CELL BIOL
PINES J, 1991, V56, P449, COLD SPRING HARB SYM
RAY LB, 1988, V85, P3753, P NATL ACAD SCI USA
ROY LM, 1990, V61, P825, CELL
SCHMANDT R, 1993, V5, P551, BAILLIERE CLIN HAEM
SCHREIBER SL, 1992, V13, P136, IMMUNOL TODAY
SHERR CJ, 1993, V73, P1059, CELL
SIGAL NH, 1992, V10, P519, ANNU REV IMMUNOL
STERN DF, 1991, V11, P987, MOL CELL BIOL
WEISS A, 1986, V4, P593, ANNU REV IMMUNOL
ZIEVE GW, 1980, V126, P397, EXP CELL RES

13/9/27 (Item 27 from file: 5)

0010010734 BIOSIS NO.: 199598478567

Regulation of intracellular signal transduction and gene expression by stress

BOOK TITLE: Neurobiological and clinical consequences of stress: From normal adaptation to post-traumatic stress disorder

AUTHOR: Duman Ronald S
BOOK AUTHOR/EDITOR: Friedman M J (Editor); Charney D S (Editor); Deutch A Y (Editor)
AUTHOR ADDRESS: Dep. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508, USA**USA

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BOOK PUBLISHER: Lippincott-Raven Publishers (a), 227 East Washington Square, Philadelphia, Pennsylvania 19106, USA
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DESCRIPTORS:

MAJOR CONCEPTS: Behavior; Biochemistry and Molecular Biophysics; Cardiovascular System--Transport and Circulation; Cell Biology; Endocrine System--Chemical Coordination and Homeostasis; Enzymology--Biochemistry and Molecular Biophysics; Genetics; Membranes--Cell Biology; Metabolism; Molecular Genetics--Biochemistry and Molecular Biophysics; Nervous System--Neural Coordination; Neurology--Human Medicine, Medical Sciences; Psychiatry--Human Medicine, Medical Sciences

BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia

ORGANISMS: human (Hominidae); rat (Muridae)

COMMON TAXONOMIC TERMS: Humans; Primates; Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates

CHEMICALS & BIOCHEMICALS: CYCLIC AMP; CYCLIC GMP; CALCIUM; NITRIC OXIDE ; ARACHIDONIC ACID; PROTEIN KINASE; **PROTEIN** TYROSINE KINASE; PROTEIN PHOSPHATASE

MISCELLANEOUS TERMS: ANIMAL MODEL; ARACHIDONIC ACID METABOLITE; BEHAVIOR; BOOK CHAPTER; BRAIN; CALCIUM; CEREBRAL CORTEX; CYCLIC AMP; CYCLIC GMP; DORSAL RAPHE; GLUCOCORTICOID; HYPOTHALAMIC-PITUITARY-ADRENAL AXIS; IMMEDIATE-EARLY GENE TRANSCRIPTION FACTOR; LOCUS COERULEUS; NERVE GROWTH FACTOR; NITRIC OXIDE; PHOSPHATIDYLINOSITOL; PHOSPHOPROTEIN; POST-TRAUMATIC STRESS DISORDER; PROTEIN PHOSPHATASE; PROTEIN PHOSPHORYLATION; PROTEIN TYROSINE KINASE;

RECEPTOR-COUPLED SECOND MESSENGER SYSTEM; SECOND MESSENGER-DEPENDENT PROTEIN KINASE; SECOND MESSENGER-INDEPENDENT PROTEIN KINASE; VENTRAL TEGMENTUM

CONCEPT CODES:
02506 Cytology - Animal
03506 Genetics - Animal
03508 Genetics - Human
07003 Behavioral biology - Animal behavior
07004 Behavioral biology - Human behavior
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10064 Biochemistry studies - Proteins, peptides and amino acids
10066 Biochemistry studies - Lipids
10067 Biochemistry studies - Sterols and steroids
10069 Biochemistry studies - Minerals
10300 Replication, transcription, translation
10506 Biophysics - Molecular properties and macromolecules
10508 Biophysics - Membrane phenomena
10612 External effects - Physical and mechanical effect
10808 Enzymes - Physiological studies
12008 Physiology - Stress
13006 Metabolism - Lipids
13008 Metabolism - Sterols and steroids
13010 Metabolism - Minerals
13012 Metabolism - Proteins, peptides and amino acids
13014 Metabolism - Nucleic acids, purines and pyrimidines
14504 Cardiovascular system - Physiology and biochemistry
17004 Endocrine - Adrenals
17014 Endocrine - Pituitary
17020 Endocrine - Neuroendocrinology
20502 Nervous system - Anatomy
20504 Nervous system - Physiology and biochemistry
20506 Nervous system - Pathology
21002 Psychiatry - Psychopathology, psychodynamics and therapy
28002 Laboratory animals - General

BIOSYSTEMATIC CODES:
86215 Hominidae
86375 Muridae

13/9/29 (Item 29 from file: 35)

01423242 ORDER NO: AADAA-I9521011

ALTERATIONS IN TYROSINE KINASE DEPENDENT SIGNAL TRANSDUCTION CORRELATE WITH FUNCTIONAL ANERGY IN T LYMPHOCYTES

Author: CHO, EUN AH
Degree: PH.D.
Year: 1994
Corporate Source/Institution: UNIVERSITY OF PENNSYLVANIA (0175)
Adviser: HELEN QUILL
Source: VOLUME 56/03-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 1334. 126 PAGES
Descriptors: HEALTH SCIENCES, IMMUNOLOGY; BIOLOGY, CELL; CHEMISTRY, BIOCHEMISTRY
Descriptor Codes: 0982; 0379; 0487

Normal CD4$\sp{+}$ Th1 clones can become functionally inactivated by ligation of T cell antigen receptor (TCR/CD3 complexes in the absence of functional antigen presenting cells (APCs). These cells show proliferative nonresponsiveness, or anergy, in response to antigen restimulation with functional APCs and this nonresponsiveness is due to the lack of IL-2 production. Recently, signal transduction through tyrosine phosphorylation has been suggested to be important in T cell activation. We have shown that anergic Th1 cells express altered levels of protein tyrosine kinases: a decrease in p56$\sp{\rm lck}$ (lck) and an increase in p59$\sp{\rm fyn}$ (fyn). Therefore, the possibility of alterations in tyrosine

phosphorylation in anergic cells upon stimulation also was analyzed. By comparing the patterns of tyrosine phosphorylation of control vs. anergic cells upon antigen restimulation, we detected significant decreases in tyrosine phosphorylation of 38kDa (p38) and 74kDa (p74) proteins in anergic cells. Defective tyrosine phosphorylation of p38 and p74 was also detected after CD3 crosslinking in anergic cells. Defective tyrosine phosphorylation of another protein, of 34kDa (p34), was found in anergic cells after CD4 crosslinking. Both the alterations in expression levels of lck and fyn and defective tyrosine phosphorylation were reversed to normal after anergic cells recovered from nonresponsiveness by growth in exogenous IL-2. In addition, normal CD4$\sp{+}$ and CD8$\sp{+}$ T cells tolerized in vivo also showed defective tyrosine phosphorylation of p38 and p74 after CD3 crosslinking. These results demonstrate that changes in tyrosine phosphorylation dependent events correlate well with the lack of IL-2 production and may be responsible for the maintenance of nonresponsiveness in anergic T cells.

13/9/33 (Item 33 from file: 155)

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Resistance of melanoma cell lines to interferons correlates with reduction of IFN-induced tyrosine phosphorylation. Induction of the anti-viral state by IFN is prevented by tyrosine kinase inhibitors.

Ralph S J; Wines B D; Payne M J; Grubb D; Hatzinisiriou I; Linnane A W; Devenish R J

Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Mar 1 1995, 154 (5) p2248-56, ISSN 0022-1767 Journal Code: 2985117R

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Clinical and experimental studies examining the action of IFNs on human malignant melanomas and melanoma cell lines have shown that this cancer cell type is frequently IFN resistant. In the present study, the IFN responsiveness of five melanoma cell lines, SK-MEL-28, SK-MEL-3, MM96, HT-144, and Hs 294T, as **determined** by the levels of IFN-induced expression of the antiviral proteins, 100 kDa 2',5'-oligoadenylate synthetase (OAS) and Mx Ag, was shown to correlate with the IFN responsiveness of the five lines **measured** in antiproliferative and antiviral **assays** . Three of the lines, SK-MEL-28 (IFN sensitive), SK-MEL-3 (moderately IFN sensitive), and MM96 (IFN insensitive) were analyzed further to ascertain their relative levels of IFN-activated **signal transduction** . Pretreatment of the three melanoma cell lines with the tyrosine kinase inhibitors, Herbimycin A or Genistein, produced a dose-dependent inhibition of the antiviral action of IFN-alpha, -beta, and -gamma and the induction of OAS by IFN-beta. Thus, induction of the antiviral state in melanoma cells by IFN requires activation of tyrosine kinase-dependent signaling pathways. Furthermore, the IFN responsiveness of three melanoma cell lines could be correlated with the ability to **detect** by immunoblotting of SDS-PAGE displays of cell lysates, IFN-induced tyrosine phosphorylated cellular proteins in the range m.w. 80 to 130 kDa. This induction was also sensitive to the tyrosine kinase inhibitors Herbimycin A and Genistein. Based on these results, we propose that the IFN-resistant melanoma cell lines examined contain a deficiency early in the IFN **signal transduction** pathway resulting in a reduced potential for IFN-induced tyrosine phosphorylation and a lack of responsiveness to IFN.

Tags: Human

Descriptors: *GTP-Binding Proteins; *Interferons--pharmacology--PD; *Melanoma--metabolism--ME; *Melanoma--therapy--TH; *Tyrosine--metabolism --ME; 2',5'-Oligoadenylate Synthetase--biosynthesis--BI; Drug Resistance;

Genistein; Isoflavones--pharmacology--PD; Melanoma--immunology--IM; Neoplasm Proteins--metabolism--ME; Phosphorylation; Protein-Tyrosine Kinase --antagonists and inhibitors--AI; Proteins--biosynthesis--BI; Quinones --pharmacology--PD; Semliki forest virus--immunology--IM; Tumor Cells, Cultured--drug effects--DE; Tumor Cells, Cultured--immunology--IM; Tumor Cells, Cultured--metabolism--ME; Virus Inhibitors--biosynthesis--BI .

CAS Registry No.: 0 (Isoflavones); 0 (Neoplasm Proteins); 0 (Proteins); 0 (Quinones); 0 (Virus Inhibitors); 0 (protein Mx); 446-72-0 (Genistein); 55520-40-6 (Tyrosine); 70563-58-5 (herbimycin); 9008-11-1 (Interferons)

Enzyme No.: EC 2.7.1.112 (Protein- **Tyrosine Kinase**); EC 2.7.7.- (2',5'-Oligoadenylate Synthetase); EC 3.6.1.- (GTP-Binding Proteins)

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Author(s): Narayanan V (REPRINT)

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Journal Subject Category: CLINICAL NEUROLOGY; PEDIATRICS

Abstract: In recent years, apoptosis, the process by which cells orchestrate their own demise, has been the subject of increasingly intense investigation, both from the stand-point of basic mechanisms of signal **transduction** and with regard to its role in **normal** and pathological processes in the nervous system, For the neurologist, an understanding of the mechanisms by which apoptosis **determines** at a cellular level the **normal** form of the nervous system, an appreciation of how both unchecked apoptosis and failure of enactment of the apoptotic pathway contribute to nervous system pathology and a sense of how both induction and inhibition of apoptosis can be exploited therapeutically are critical to applying the basic knowledge in this field to human disease, Early studies made it clear that substances produced by the target tissue influenced the survival of developing neurons, More recent investigations have demonstrated that they do so by influencing the production of a series of endogenous mediators and modulators of neuronal survival. Furthermore, it is evident that apoptosis is important for the development of both neuronal and non-neuronal cells in the peripheral and central nervous systems.

Identifiers--KeyWord Plus(R): EMBRYO SPINAL-CORD; INDUCED NEURONAL DEATH; CHICK-EMBRYO; NEUROTROPHIC FACTOR; GROWTH-FACTOR; MOTONEURON SURVIVAL; SYMPATHETIC NEURONS; MOTOR-NEURONS; RNA-SYNTHESIS; IN-VITRO

Research Fronts: 95-4914 002 (PROGRAMMED NEURONAL DEATH; APOPTOSIS IN NEURAL CELLS; BRAIN-DERIVED NEUROTROPHIC FACTOR SURVIVAL RESPONSE)

95-0477 001 (CYTOKINE RECEPTOR SIGNALING MECHANISMS; ACTIVATION OF MULTIPLE **PROTEIN - TYROSINE KINASES** ; STAT TRANSCRIPTION FACTORS; EARLY RESPONSE GENES)

95-1680 001 (PROGRAMMED CELL-DEATH; BCL-2 GENE FAMILY; REGULATION OF APOPTOSIS)

95-2493 001 (LOW-AFFINITY NEUROTROPHIN RECEPTORS; CULTURED BASAL FOREBRAIN CHOLINERGIC NEURONS; TRKA EXPRESSION; NGF TRANSGENIC MICE; NERVE GROWTH-FACTOR)

Cited References:
BARBIN G, 1984, V43, P1468, J NEUROCHEM
BARDE YA, 1982, V1, P549, EMBO J
BARRES BA, 1992, V23, P1221, J NEUROBIOL
BARRETT GL, 1994, V91, P6501, P NATL ACAD SCI USA
BREDESEN DE, 1995, V38, P839, ANN NEUROL
CAJAL SRY, 1929, P400, STUDIES VERTEBRATE N
CHUWANG IW, 1978, V177, P59, J COMP NEUROL
COWAN WM, 1973, P19, DEV AGING NERVOUS SY
COWAN WM, 1984, V225, P1258, SCIENCE
GAGLIARDINI V, 1994, V261, P826, SCIENCE
GARCIA I, 1992, V258, P302, SCIENCE
GREENLUND LJS, 1995, V14, P303, NEURON
HAMBURGER V, 1975, V160, P535, J COMP NEUROL
HAMBURGER V, 1949, V111, P457, J EXP ZOOL
HAMBURGER V, 1992, V23, P1116, J NEUROBIOL
HAMBURGER V, 1981, V1, P60, J NEUROSCI
HAMBURGER V, 1984, V4, P767, J NEUROSCI
HOLLYDAY M, 1976, V170, P311, J COMP NEUROL
HOUENOU LJ, 1995, V92, P895, P NATL ACAD SCI USA
LEVIMONTALCINI R, 1950, V86, P253, J MORPHOL
LEVIMONTALCINI R, 1960, V42, P384, P NATL ACAD SCI USA
LEVIMONTALCINI R, 1968, V48, P534, PHYSIOL REV
LIN LFH, 1993, V260, P1130, SCIENCE
MARTIN DP, 1988, V106, P829, J CELL BIOL
MARTIN DP, 1992, V23, P1205, J NEUROBIOL
MILLIGAN CE, 1994, V25, P1005, J NEUROBIOL
MILLIGAN CE, 1995, V15, P385, NEURON
NEFF NT, 1993, V24, P1578, J NEUROBIOL
OKADO N, 1984, V4, P1639, J NEUROSCI
OPPENHEIM RW, 1991, V14, P453, ANN REV NEUROSCI
OPPENHEIM RW, 1977, V125, P154, BRAIN RES
OPPENHEIM RW, 1990, V138, P104, DEV BIOL
OPPENHEIM RW, 1978, V177, P87, J COMP NEUROL
OPPENHEIM RW, 1992, V23, P1111, J NEUROBIOL
OPPENHEIM RW, 1993, V24, P1065, J NEUROBIOL
OPPENHEIM RW, 1982, V295, P57, NATURE
OPPENHEIM RW, 1992, V360, P755, NATURE
OPPENHEIM RW, 1995, V373, P344, NATURE
OPPENHEIM RW, 1988, V240, P919, SCIENCE
OPPENHEIM RW, 1991, V251, P1616, SCIENCE
OPPENHEIM RW, 1981, P74, STUDIES DEV NEUROBIO
OPPENHEIM RW, 1985, V8, P487, TRENDS NEUROSCI
PITTMAN R, 1979, V187, P425, J COMP NEUROL
PITTMAN RH, 1978, V271, P364, NATURE
SENDTNER M, 1992, V360, P757, NATURE
SNIDER WD, 1989, V26, P489, ANN NEUROL
THORNBERRY NA, 1992, V356, P768, NATURE
YAN Q, 1992, V360, P753, NATURE
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12763985 PMID: 7554458

Nonreceptor protein tyrosine kinase involvement in signal transduction and immunodeficiency disease .

Saouaf S J; Burkhardt A L; Bolen J B

Department of Molecular Oncology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543, USA.

Clinical immunology and immunopathology (UNITED STATES) Sep 1995, 76 (3 Pt 2) pS151-7, ISSN 0090-1229 Journal Code: 0356637

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The nonreceptor **protein tyrosine kinases** (PTKs) have been grouped into 10 different enzyme families based on predicted amino acid sequences. As the number of enzymes belonging to the nonreceptor class of PTK is increasing, one challenge is to **determine** how these various classes of PTKs interact within the cell to promote **signal transduction** . Herein, the activation of four classes of nonreceptor PTKs is discussed in relation to their interactions with each other as well as with other signaling molecules during the process of lymphocyte surface antigen receptor-mediated activation. Recent findings of nonreceptor PTK loss-of-function mutations in different immunodeficiency diseases has revealed the important contribution of this group of enzymes to lymphocyte development. (65 Refs.)

Tags: Human

Descriptors: *Immunologic Deficiency Syndromes--enzymology--EN; *Protein-Tyrosine Kinase--physiology--PH; Amino Acid Sequence; Animals; Molecular Sequence Data; Signal Transduction--physiology--PH

Enzyme No.: EC 2.7.1.112 (**Protein - Tyrosine Kinase**)

Record Date Created: 19951024

Record Date Completed: 19951024

13/9/10 (Item 10 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

12804816 PMID: 7486682

Signal transduction interception as a novel approach to disease management.

Levitzki A

Department of Biological Chemistry, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Israel.

Annals of the New York Academy of Sciences (UNITED STATES) Sep 7 1995, 766 p363-8, ISSN 0077-8923 Journal Code: 7506858

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

(22 Refs.)

Tags: Human

Descriptors: *Enzyme Inhibitors--therapeutic use--TU; *Protein- **Tyrosine** Kinase--antagonists and inhibitors--AI; *Signal Transduction; *Therapeutics; Animals; Anti-Inflammatory Agents, Non-Steroidal --therapeutic use--TU; Anti-Inflammatory Agents, Non-Steroidal--toxicity --TO; Antineoplastic Agents--therapeutic use--TU; Antineoplastic Agents --toxicity--TO; Arteriosclerosis--prevention and control--PC; Drug Design; Enzyme Inhibitors--toxicity--TO; Neovascularization, Pathologic--prevention and control--PC; Recurrence

CAS Registry No.: 0 (Anti-Inflammatory Agents, Non-Steroidal); 0 (Antineoplastic Agents); 0 (Enzyme Inhibitors)

Enzyme No.: EC 2.7.1.112 (Protein-Tyrosine Kinase)

Record Date Created: 19951206

Record Date Completed: 19951206

13421233 PMID: 9120025

Cytokine signaling through the novel tyrosine kinase RAFTK in Kaposi's sarcoma cells.

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Journal of clinical investigation (UNITED STATES) Apr 1 1997, 99 (7) p1798-804, ISSN 0021-9738 Journal Code: 7802877

Contract/Grant No.: HL-43510-07; HL; NHLBI; HL-53745-02; HL; NHLBI; HL-55187-01; HL; NHLBI; +

Document type: Journal Article

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Main Citation Owner: NLM

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Subfile: AIM; INDEX MEDICUS

A number of cytokines, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), oncostatin M (OSM), IL-6, and tumor necrosis factor alpha (TNF-alpha), have been postulated to have a role in the pathogenesis of Kaposi's sarcoma (KS). The proliferative effects of bFGF and OSM may be via their reported activation of the c-Jun NH2-terminal kinase (JNK) signaling pathway in KS cells. We now report that KS cells express a recently identified focal adhesion kinase termed RAFTK which appears in other cell systems to coordinate surface signals between cytokine and integrin receptors and the cytoskeleton as well as act downstream to modulate JNK activation. We also report that the tyrosine kinase receptor FLT-4, present on normal lymphatic endothelium, is robustly expressed in KS cells. Treatment of KS cells with VEGF-related protein (VRP), the ligand for the FLT-4 receptor, as well as with the cytokines bFGF, OSM, IL-6, VEGF, or TNF-alpha resulted in phosphorylation and activation of RAFTK. Following its activation, there was an enhanced association of RAFTK with the cytoskeletal protein paxillin. This association was mediated by the hydrophobic COOH-terminal domain of the kinase. Furthermore, JNK activity was increased in KS cells after VEGF or VRP stimulation. We postulate that in these tumor cells RAFTK may be activated by a diverse group of stimulatory cytokines and facilitate signal transduction to the cytoskeleton and downstream to the growth promoting JNK pathway.

Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Cytokines--pharmacology--PD; *Mitogen-Activated Protein Kinase Kinases; *Protein-Tyrosine Kinase--physiology--PH; *Sarcoma, Kaposi --metabolism--ME; Animals; Cytoskeletal Proteins--metabolism--ME; Phosphoproteins--metabolism--ME; Phosphorylation; Protein Kinases --metabolism--ME; Rabbits; Receptor Protein-Tyrosine Kinases--analysis--AN; Receptors, Growth Factor--analysis--AN; Receptors, Vascular Endothelial Growth Factor; Sarcoma, Kaposi--pathology--PA; Tumor Cells, Cultured

CAS Registry No.: 0 (Cytokines); 0 (Cytoskeletal Proteins); 0 (Phosphoproteins); 0 (Receptors, Growth Factor); 0 (paxillin)

Enzyme No.: EC 2.7.1.- (JNK-activating protein kinase); EC 2.7.1.- (protein tyrosine kinase **PYK2**); EC 2.7.1.112 (Protein-Tyrosine Kinase) ; EC 2.7.1.112 (Receptor Protein-Tyrosine Kinases); EC 2.7.1.112 (Receptors, Vascular Endothelial Growth Factor); EC 2.7.1.37 (Mitogen-Activated Protein Kinase Kinases); EC 2.7.1.37 (Protein Kinases)

Record Date Created: 19970424

Cytokine Signaling Through the Novel Tyrosine Kinase RAFTK in Kaposi's Sarcoma Cells

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Abstract

A number of cytokines, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), oncostatin M (OSM), IL-6, and tumor necrosis factor alpha (TNF-α), have been postulated to have a role in the pathogenesis of Kaposi's sarcoma (KS). The proliferative effects of bFGF and OSM may be via their reported activation of the c-Jun NH_2-terminal kinase (JNK) signaling pathway in KS cells. We now report that KS cells express a recently identified focal adhesion kinase termed RAFTK which appears in other cell systems to coordinate surface signals between cytokine and integrin receptors and the cytoskeleton as well as act downstream to modulate JNK activation. We also report that the tyrosine kinase receptor FLT-4, present on normal lymphatic endothelium, is robustly expressed in KS cells. Treatment of KS cells with VEGF-related protein (VRP), the ligand for the FLT-4 receptor, as well as with the cytokines bFGF, OSM, IL-6, VEGF, or TNF-α resulted in phosphorylation and activation of RAFTK. Following its activation, there was an enhanced association of RAFTK with the cytoskeletal protein paxillin. This association was mediated by the hydrophobic COOH-terminal domain of the kinase. Furthermore, JNK activity was increased in KS cells after VEGF or VRP stimulation. We postulate that in these tumor cells RAFTK may be activated by a diverse group of stimulatory cytokines and facilitate signal transduction to the cytoskeleton and downstream to the growth promoting JNK pathway. (*J. Clin. Invest.* 1997. 99:1798–1804.) Key words: RAFTK, related adhesion focal tyrosine kinase • Kaposi's sarcoma (KS) • cytokines

Introduction

Kaposi's sarcoma (KS)[1] is the most frequently observed neoplasm arising among patients with AIDS. The cell of origin is believed to be from the lymphatic endothelium (1–2). Etiological factors implicated in KS include the recently discovered human herpesvirus 8 (HHV-8)/Kaposi's sarcoma herpesvirus (KSHV) and TAT, the soluble transcriptional activator of HIV (3–7). Considerable data indicate a role for endogenous and exogenous cytokines in the pathogenesis of KS (8–16). Growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), which are known to stimulate the mitogenesis of certain types of endothelium, as well as Oncostatin M (OSM), IL-6, and tumor necrosis factor alpha (TNF-α) which are elaborated during inflammatory conditions, have been implicated in promoting KS cell growth (17–25).

Defining the signal transduction pathways which may be utilized by cytokines that appear to modulate KS growth provides an opportunity for the rational and targeted therapeutic intervention against this neoplasm. One issue that is immediately apparent is that the cytokines described to date as promoting KS belong to distinctly different families as defined by their receptors. VEGF and bFGF receptors belong to the protein tyrosine kinase family, OSM and IL-6 utilize a common gp130 subunit, and TNF-α receptors are members of the Fas/apoptosis CD95 family. Given this diversity, we have further characterized the downstream signaling pathways triggered by cytokine treatment in permanent human KS cells in vitro and sought a common molecule among these different pathways. We observed that KS cells express a newly identified signaling molecule termed related adhesion focal tyrosine kinase (RAFTK), also known as Pyk2 or CAK-β (26–29). RAFTK is a member of the focal adhesion kinase (FAK) family and has considerable deduced amino acid and structural similarity with FAK. Previously, RAFTK has been reported to link calcium and integrin-mediated signaling to the cytoskeleton in brain and hematopoietic cells (26–29).

In KS cells, we find that treatment with cytokines from different families, including bFGF, OSM, IL-6, VEGF, and TNF-α, all led to the phosphorylation and activation of RAFTK. After cytokine treatment, RAFTK was found to associate with the cytoskeletal protein paxillin. We have extended this observation and focused on the tyrosine kinase receptor termed FLT-4, which has been found in fetal and adult lymphatic endothelium (30–32). KS cells expressed the FLT-4 receptor, and treatment with its newly discovered ligand called VEGF-

The first two authors, Z.Y. Liu and R.K. Ganju contributed equally to this work.

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1. *Abbreviations used in this paper:* bFGF, basic fibroblast growth factor; GST, glutathione *S*-transferase; JNK, c-Jun NH_2-terminal kinase; KS, Kaposi's sarcoma; OSM, Oncostatin M; RAFTK, related adhesion focal tyrosine kinase; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; VRP, VEGF-related protein.

related protein (VRP) or VEGF-C (33–34), again resulted in the phosphorylation of RAFTK.

Recent studies have identified the c-Jun NH_2-terminal kinase (JNK), also known as stress-activated protein kinase (SAPK), is involved in signaling events downstream of RAFTK/Pyk2 activation (35). The JNK kinases have also been shown to participate in cellular proliferation via transcriptional activation (36–38). Since OSM previously has been shown to activate JNK in KS cells (39), we studied the activation of JNK by VEGF and VRP in KS to better characterize signaling pathways from linking cytokine receptors to events involved in transcriptional activation.

Methods

Cells and cell culture. The human KS cell line KS 59 was derived from the cutaneous biopsy of an AIDS patient as previously described (10, 40–42). Similarly, a different KS cell line, KS 38, was derived from a biopsy of a cutaneous lesion from another AIDS patient. These cell lines possess many characteristics of spindle cells in primary KS lesions and are positive for von Willebrand's factor and smooth muscle antigens. The cells were grown on 1.5% gelatin-coated flasks and were carried in RPMI 1640 with 15% FCS, 2 mM glutamine, 1 mM MEM sodium pyruvate, 0.05 mM MEM non-essential amino acids, 1× MEM amino acids, 1% Nutridoma-HU (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 50 μg/ml penicillin and 50 μg/ml streptomycin. Cultures were carried until near confluence prior to the different treatments in the signaling studies described below. 293T cells (human transformed primary embryonal kidney 293T cells) were transfected with the FLT-4 gene and used as controls for the detection of the receptor protein as described (33). CMK megakaryocytic cells were used as a positive control for RAFTK expression (29) and Jurkat T-cells as a negative control for FLT-4 expression (30).

Reagents and antibodies. RAFTK antibodies were generated using glutathione *S*-transferase (GST)-fusion proteins to the various domains of the molecule and by immunizing New Zealand rabbits as previously described (26, 29). Using an ELISA assay, sera were screened for specific binding to RAFTK. Serum R-4250 was chosen for further studies based on its titer in the ELISA. Serum R-4250 did not cross-react with FAK and was specific for RAFTK. Antibodies to the VEGF receptor FLK-1, the FLT-4 receptor, paxillin, JNK as well as the recombinant GST-c-Jun NH_2-protein (1–79 amino acids) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal anti-phosphotyrosine antibody (4G10) was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Electrophoresis reagents were obtained from Bio-Rad Laboratories (Hercules, CA). Phorbol 12-myristate 13-acetate (PMA), the protease inhibitors leupeptin, aprotinin, and alpha 1 antitrypsin, and all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). The recombinant cytokines bFGF, TNF-α, and IL-6 were obtained from R&D Systems, Inc. (Minneapolis, MN). Recombinant VRP, the ligand for the FLT-4 receptor, was expressed and purified from a glioblastoma cell line as previously reported (33). Recombinant VEGF was obtained from Genentech, Inc. (South San Francisco, CA). Recombinant OSM was obtained from the AIDS Reagent Bank (Bethesda, MD).

Indirect immunofluorescence. KS 38 or KS 59 cells were cultured in Chamber Slides (Lab-tek, Naperville, IL) to 90% confluency, and were then washed twice with ice-cold PBS and fixed for 30 min in 4% paraformaldehyde. Cells were next washed three times with ice-cold PBS and blocked for nonspecific staining using 10% FCS in PBS for 30 min on ice. FLT-4 and FLK-1 expression were determined using purified antiserum at a 1:100 dilution for 1 h on ice. Normal rabbit serum (NRS) was used as a control for nonspecific staining. After washing cells three times with PBS, cells were stained with secondary antibody conjugated to FITC (Boehringer Mannheim Biochemicals) at a 1:500 dilution for 1 h on ice. Proteins were visualized and photographed after washing three times with PBS using an inverted fluorescence microscope.

Stimulation of cells. KS 38 or KS 59 cells, grown to 80% confluence, were serum-starved for 16–18 h and washed twice with HBSS (GIBCO BRL, Gaithersburg, MD) prior to PMA or cytokine treatments. KS cells were first treated with PMA to assess the effects of this chemical stimulus known to phosphorylate RAFTK in other cell systems (26–29). After a time course of stimulation with PMA was established, the effects of the various cytokines were studied. We treated cells with 100 ng/ml of VEGF, VRP, or bFGF adding 10 IU/ml of heparin in each case. Treatment with 100 ng/ml of TNF-α, OSM, or IL-6 was done in the absence of heparin. Cytokines were added to cultures singly for different time periods in vitro. Controls included media with or without 10 IU/ml heparin in the absence of cytokines. After stimulation, cell lysates were directly prepared within the culture dish by lysis in 500 μl modified RIPA buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM PMSF, 10 μg/ml of aprotinin, leupeptin and pepstatin, 10 mM sodium vanadate, 10 mM sodium fluoride and 10 mM sodium pyrophosphate) per dish at varying timepoints. Total cell lysates (TCL) were clarified by centrifugation at 10,000 *g* for 10 min. Protein concentrations were determined by protein assay (Bio-Rad Laboratories).

Immunoprecipitation and Western blot analysis. For the immunoprecipitation studies, identical amounts of protein from each sample were clarified by incubation with protein A-sepharose CL-4B (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) for 1 h at 4°C. Following the removal of the protein A-sepharose by brief centrifugation, the solution was incubated with different primary antibodies as detailed below for each experiment for 4 h or overnight at 4°C. The immunoprecipitations of the antibody–antigen complexes were performed by incubation for 2 h at 4°C with 75 μl of the protein A-sepharose (10% suspension). Nonspecific bound proteins were removed by washing the sepharose beads three times with the modified RIPA buffer and one time with PBS. Bound proteins were solubilized in 40 μl of 2× Laemmli buffer and further analyzed by immunoblotting. Samples were separated on 7.5% SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat milk protein and probed with primary antibody for 2 h at RT or 4°C overnight. Immunoreactive bands were visualized using HRP-conjugated secondary antibody and the enhanced chemiluminescent (ECL) system (Amersham Corp., Arlington Heights, IL).

In vitro GST-fusion protein RAFTK C-terminal association assay. The GST-fusion protein of the RAFTK COOH-terminal domain (amino acids 681–1,009) was amplified by the PCR technique and cloned into the pGEX-2T expression vector (Pharmacia LKB Biotechnology, Inc.) as previously described (29). The GST-fusion protein was produced by 1 mM isopropyl β-thiogalactopyranoside induction and purified by affinity chromatography on a glutathione-sepharose column according to the manufacturer's recommendations (Pharmacia LKB Biotechnology, Inc.).

For the in vitro association experiments, TCL (1 mg) of the stimulated KS cells were mixed with 5 μg of the purified GST-fusion protein of the RAFTK C-terminal domain (29) and incubated for 1 h at 4°C on a rotatory shaker. 50 μl of the glutathione-sepharose 4B beads (Pharmacia LKB Biotechnology, Inc.) were added to preabsorb the complex. After incubation for 3 h at 4°C on a rotatory shaker, the beads were centrifuged and washed three times with the modified RIPA buffer. The bound proteins were eluted by boiling in Laemmli sample buffer and subjected to SDS-PAGE on a 7.5% gel and to Western blot analysis.

JNK kinase assay. Cell lysates were immunoprecipitated with JNK antibody (Santa Cruz Biotechnology). The immune complexes were washed twice with RIPA buffer and once in kinase buffer (50 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 100 μM ATP). The complex was then incubated in kinase buffer containing recombinant GST c-Jun

0.2 μg/μl (1–79 amino acids) (Santa Cruz Biotechnology) and 5 μCi [γ^{32}P]ATP for 30 min at RT. The reaction was terminated by adding 2× SDS sample buffer and boiling the sample for 5 min at 100°C. Proteins were separated on 12% SDS-PAGE and detected by autoradiography. Rabbit IgG was used as a negative control.

In vitro kinase assay. Cell lysates were immunoprecipitated with RAFTK antiserum. The immunoprecipitated complexes were washed twice with RIPA buffer and once in kinase buffer (20 mM Hepes, pH 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, and 100 mM Na_3VO_4). The immune complex was incubated in kinase buffer containing 25 μg of poly (Glu/Tyr) (4:1, 20–50 kD; Sigma Chemical Co.) and 5 μCi [γ^{32}P]ATP at RT for 30 min. The reaction was stopped by adding 2× SDS sample buffer and boiling the sample for 5 min at 100°C. Proteins were then separated on 7.5% SDS-PAGE and detected by autoradiography. Normal rabbit serum was used as a negative control.

Results

Kaposi's Sarcoma cells express FLK-1 and FLT-4 receptors. To characterize the effects of different cytokines on KS cell signaling, we first examined the KS 38 and KS 59 cell lines for the expression of receptors for members of the VEGF family. Because KS spindle cells appear to be derived from the lymphatic endothelium, we focused on the recently identified FLT-4 receptor as well as on the VEGF receptor FLK-1 (30–34, 43). Using indirect immunofluorescence, the expression of both the FLK-1 receptor and the FLT-4 receptor was readily observed (data not shown). The presence of the FLT-4 receptor was confirmed by Western blot analysis using a specific FLT-4 polyclonal antibody (Fig. 1 *A*).

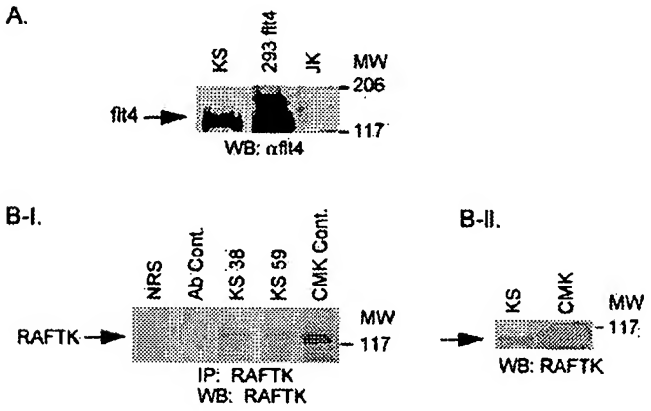

Figure 1. (*A*) Expression of FLT-4 in KS 38 cells. TCL from KS 38 cells were resolved on 7.5% SDS-PAGE, transferred onto a nitrocellulose membrane and immunoblotted with the anti–FLT-4 antibody. TCL from human embryonic kidney 293T cells transfected with FLT-4 were used as a positive control and Jurkat (JK) T cells were used as a negative control. (*B*) Expression of RAFTK in KS 38 and KS 59 cells detected by immunoprecipitation or Western blot analysis. TCL (1 mg) from KS 38 or KS 59 cells were immunoprecipitated with the anti-RAFTK antibody, using the same amount of cell lysate from CMK megakaryocytic cells as a positive control. Immunoprecipitates or TCL were size-fractionated on 7.5% SDS-PAGE, transferred onto a nitrocellulose membrane and then immunoblotted with anti–RAFTK antibody. NRS and antibody alone were used as negative controls (*B-I*). TCL (100 mg) from KS 38 cells were size-fractionated on 7.5% SDS-PAGE, transferred onto a nitrocellulose membrane and then immunoblotted with anti–RAFTK antibody. The same amount of total cell lysate from the CMK megakaryocytic cells was used as a positive control for RAFTK (*B-II*).

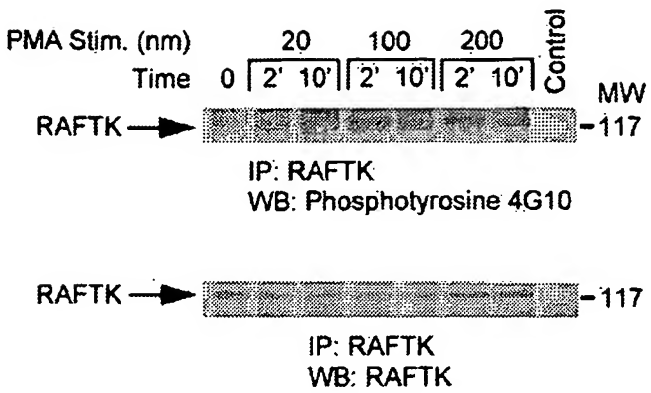

Figure 2. Tyrosine phosphorylation of RAFTK in KS 38 cells by PMA stimulation. Starved KS 38 cells were treated with the indicated concentration of PMA for 2 or 10 min. TCL from treated or untreated cells were immunoprecipitated with the anti–RAFTK antibody. Immunoprecipitates were size-fractionated on 7.5% SDS-PAGE, and subjected to serial immunoblotting with anti–phosphotyrosine antibody (*top*) and anti–RAFTK antibody (*bottom*). Antibody alone was used as a negative control.

RAFTK is expressed in Kaposi's Sarcoma cells and phosphorylated upon cytokine treatment. We proceeded to further characterize the KS cells for the expression of RAFTK, a molecule that appears to be activated in brain and hematopoietic cells and reported previously to link surface signals to the JNK signaling pathway (26–29, 35). As shown in Fig. 1, *B-I* and *B-II*, KS 38 or KS 59 cells expressed significant amounts of RAFTK protein as detected by Western blotting and immunoprecipitation.

Treatment of KS 38 cells with the chemical inducer PMA resulted in a time-dependent phosphorylation of RAFTK (Fig. 2). Similar results were observed with KS 59 cells (data not shown), so that all subsequent experiments were performed in KS 38 cells. Having established that RAFTK is expressed and can be phosphorylated in KS 38 cells, we studied whether the treatment of these cells with VEGF or VRP, respective ligands for the FLK-1 and FLT-4 receptors, would result in the activation of RAFTK signaling pathways. As shown in Fig. 3 *A*, there was a clear time-dependent phosphorylation of RAFTK in response to VRP. Similar changes were observed following treatment with VEGF. We noted some fluctuation in the phosphorylation of RAFTK over this time course of treatment, which may represent the activity of endogenous phosphatases.

Previously, cytokines such as bFGF, OSM, IL-6, and TNF-α have been reported to promote the in vitro proliferation of KS cells. Thus, we analyzed the effects of treatment with these cytokines on RAFTK phosphorylation in KS 38 cells. As shown in Fig. 3, *B–D*, each of these cytokines resulted in the phosphorylation of this novel tyrosine kinase. In each of these studies, we observed changes in RAFTK phosphorylation without alteration of RAFTK protein levels (see bottom panels, Fig. 3, *A–D*). As seen in other studies (29), RAFTK was detected as a single or double band depending on the resolution of the gels.

Cytokine treatment of Kaposi's Sarcoma cells enhances RAFTK association with the cytoskeletal protein paxillin. Following our observation that RAFTK was phosphorylated by

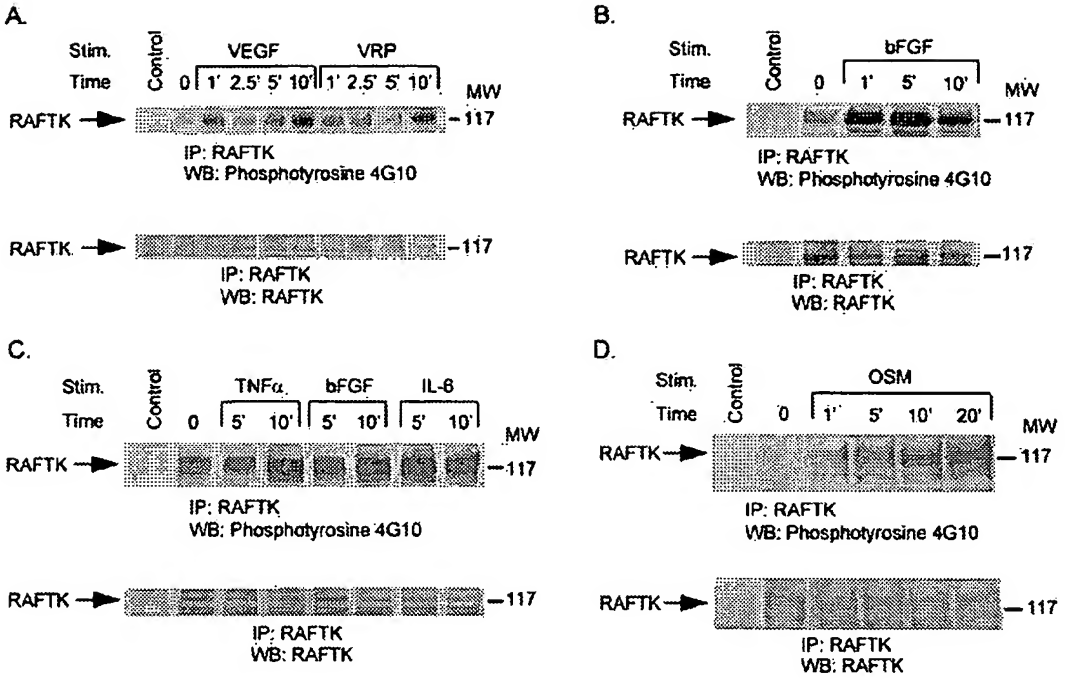

Figure 3. Tyrosine phosphorylation of RAFTK in KS 38 cells by cytokine treatment. KS 38 cells were serum-starved overnight and then treated with VEGF (100 ng/ml + 10 IU/ml heparin) or VRP (100 ng/ml + 10 IU/ml heparin) (*A*); bFGF (100 ng/ml + 10 IU/ml heparin) alone (*B*); TNF-α (100 ng/ml) or IL-6 (100 ng/ml) with bFGF as a positive control (*C*); and OSM (100 ng/ml) (*D*) for the indicated time period. TCL (1 mg) from treated or untreated cells were immunoprecipitated with the anti–RAFTK antibody. Immunoprecipitates were resolved on 7.5% SDS-PAGE and subjected to serial immunoblotting with the anti–phosphotyrosine antibody (*top*) and anti–RAFTK antibody (*bottom*). NRS was used as a negative control.

the cytokines OSM, IL-6, bFGF, and TNF-α which are known to stimulate KS cell growth, as well as the endothelial growth factors VEGF and VRP which modulate cell permeability (43) and migration (33–34), we asked if this phosphorylation might modulate the association of RAFTK with certain cytoskeletal molecules. Previously, we have seen colocalization of RAFTK with vinculin in human megakaryocytes by confocal microscopy (29). Using RAFTK immunoprecipitation followed by immunoblotting with antipaxillin antibodies, we found an association of these two molecules which increased following cytokine treatments at different time points (Fig. 4, *A* and *B*).

There was no uniform kinetics to this association following the different cytokine treatments. To further characterize this association, we immunoprecipitated with the COOH-terminal domain of RAFTK and immunoblotted with antipaxillin antibodies. As shown in Fig. 5, paxillin associated with the COOH

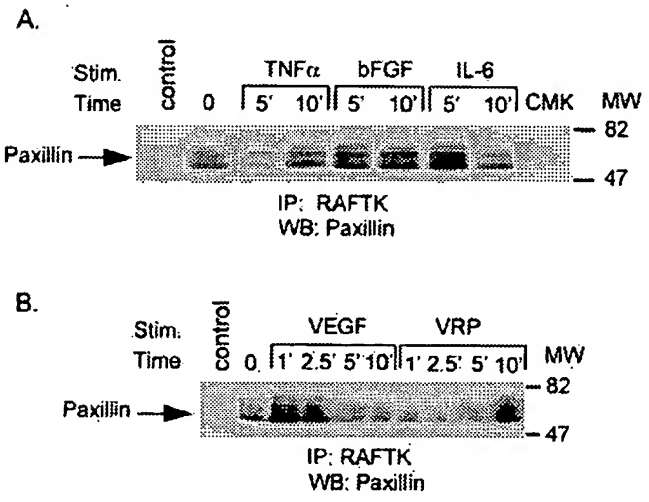

Figure 4. Association of RAFTK with paxillin in cytokine treated KS 38 cells. Cell lysates from KS 38 cells treated with TNF-α, bFGF or IL-6 (*A*), or VEGF or VRP (*B*) were immunoprecipitated by the anti–RAFTK antibody. The immunoprecipitates were resolved on 7.5% SDS-PAGE, transferred onto a nitrocellulose membrane and immunoblotted with the anti–paxillin antibody. NRS was used as a negative control.

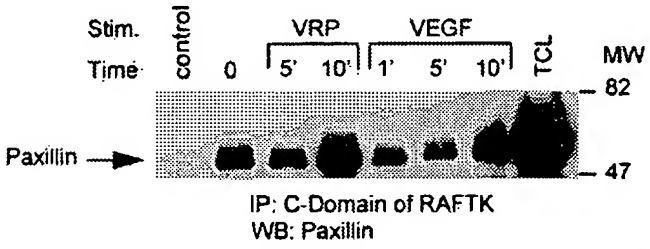

Figure 5. Association of the GST-fusion protein RAFTK COOH-terminal domain with paxillin. Unstimulated or stimulated KS 38 cell lysates were immunoprecipitated with the GST-fusion protein RAFTK C-terminal domain, separated on 7.5% SDS-PAGE, transferred onto a nitrocellulose membrane and immunoblotted with the anti–paxillin antibody. TCL with Glutathione Sepharose beads were used as a negative control.

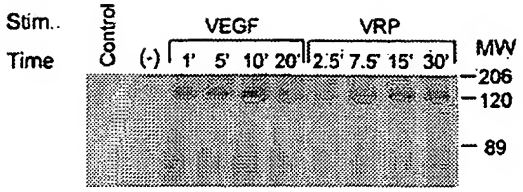

Figure 6. Stimulation of in vitro tyrosine kinase activity of RAFTK following VEGF or VRP treatment. Unstimulated or stimulated KS cell lysates were immunoprecipitated with RAFTK antibody. The immune complex was incubated with kinase buffer containing 25 μg of poly (Glu/Tyr) (4:1) and 5 μCi [γ^{32}P]ATP at RT for 30 min. The ^{32}P-incorporated proteins were resolved on 7.5% SDS-PAGE followed by autoradiography.

terminus of RAFTK. This association increased following VRP or VEGF stimulation.

Cytokine stimulation activates the protein tyrosine kinase activity of RAFTK. The tyrosine phosphorylation of protein tyrosine kinases can result in the activation of its kinase activity, which is essential for its role in signal transduction. We therefore performed an in vitro kinase assay in which poly (Glu/Tyr) (4:1) was used as an exogenous substrate to determine the intrinsic tyrosine kinase activity of RAFTK. As shown in Fig. 6, VEGF or VRP stimulation of KS cells resulted in the activation of the intrinsic tyrosine kinase activity of RAFTK. However, the kinetics of this kinase activation were different from those of the total tyrosine phosphorylation of RAFTK. This difference may be a result of the use of an exogenous substrate.

Activation of JNK activity by OSM, VEGF, and VRP. JNK is a novel member of the mitogen-activated protein kinase (MAPK) family, and has recently been shown to be a downstream mediator of the RAFTK/Pyk2 signaling pathway.

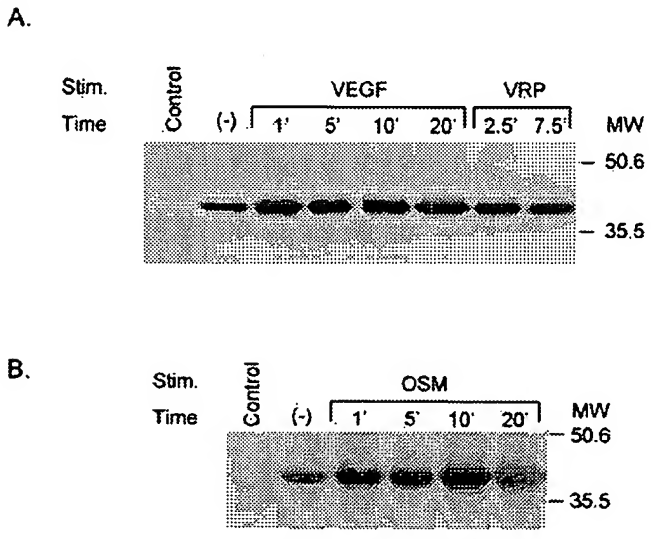

Figure 7. VEGF and VRP activate the JNK kinase in KS 38 cells. Unstimulated or stimulated KS 38 cell lysates were immunoprecipitated with the anti-JNK antibody. The immune complex was incubated with kinase buffer containing 5 μCi [γ^{32}P]ATP and 0.2 μg/μl GST-c-Jun at RT for 30 min. The reaction products were resolved on 12% SDS-PAGE and detected by autoradiography.

Previously, OSM and bFGF have been reported to increase JNK activity in KS cells (39). We therefore sought to determine if other cytokines which we observed to stimulate RAFTK phosphorylation and activity also activated JNK in KS cells. As shown in Fig. 7 *A*, VEGF and VRP stimulation of KS cells resulted in the activation of JNK as determined by the phosphorylation of GST–c-Jun (1–79 amino acids). Earlier studies using GST–c-Jun immunoprecipitates showed that OSM treatment increased JNK activity in KS cells (39). In this study, we also found an increase in JNK activity in anti-JNK immunoprecipitates upon OSM treatment (Fig. 7 *B*).

Discussion

The development of authentic permanent KS cell lines has afforded the opportunity to characterize the surface structures of these cells and to examine which cytokines may modulate their proliferation. There is extensive literature supporting a role for a number of cytokines in modulating KS cell growth via autocrine or paracrine mechanisms (8–20). The characterization of signaling pathways in KS cells and the effects of these cytokines on such pathways have been less extensively explored. Amaral et al. (44) found that OSM activated the MAP kinase pathway, while Faris et al. (39) reported that members of the Jak/Stat family of kinases known to participate in signaling via the gp130 receptor were active in KS cells as well. In our studies, we chose the KS 38 cell line derived from a patient with cutaneous KS as a model because of its previously characterized properties that closely correspond to those of primary pathological KS specimens (40, 41). However, there are limitations within in vitro cell models, particularly the lack of infection of KSHV. We sought to identify on these KS cells novel receptors which are preferentially expressed in normal lymphatic endothelium, and to further characterize the signaling pathways that may link surface receptor activation to the cytoskeleton and transcriptional activation in these cells.

The tyrosine kinase FLT-4 receptor is relatively restricted in expression in normal tissues, with prior studies indicating its presence on the surface of the lymphatic endothelium as well as microvascular endothelial cells (30–32). We observed that KS 38 cells, whose characteristics resemble those of spindle cells in primary lesions, express FLT-4 as well as the related FLK-1 receptor. We also observed that VRP, the ligand for the FLT-4 receptor, as well as VEGF, the ligand for FLK-1, induced significant signaling changes in KS 38 cells based on the enhanced phosphorylation of their proteins. After this observation, we sought to identify whether or not there are shared signaling molecules whose enhanced phosphorylation is a common response to these cytokines as well as those cytokines previously reported to stimulate KS cells.

We were aware that a variety of ligands and receptors from different molecular families have been implicated in the pathogenesis of KS. To survey this range of cytokines, we chose representative cytokines from each family and performed a comparative analysis of bFGF, TNF-α, OSM, and IL-6 with VEGF and VRP. We found that in all these diverse cytokine signaling pathways, the recently identified RAFTK molecule was phosphorylated and its kinase activity enhanced.

RAFTK appears to function as a platform kinase upon which a number of intracytoplasmic kinases and adapter molecules converge. The convergence of such molecules likely facilitates the transmission of surface signals to the cytoskeleton.

Previous studies from several laboratories including our own indicate a role for RAFTK/Pyk2 in calcium-mediated signaling in brain as well as signaling by integrins and certain cytokines such as the stem cell factor/kit ligand in megakaryocytes (26–29). Recently, RAFTK/Pyk2 was shown to couple with the JNK signaling pathway in PC12 neuronal and 293T kidney cells upon its activation by stress signals (35). JNK activation is a pivotal step in the regulation of certain transcriptional mediators such as AP-1 (45).

In this study, we report that after RAFTK activation by these diverse cytokines there was enhanced association of this kinase with the cytoskeletal protein paxillin. Previously, in megakaryocytes, RAFTK was seen to colocalize with vinculin, another cytoskeletal protein (29). In KS 38 cells, the association of the kinase with paxillin appeared to be mediated by the proline-rich COOH-terminal domain of RAFTK. This interaction may contribute to cytokine modulation of KS cell migration and/or adhesion, as is suggested by the recent report of RAFTK's association with paxillin in hematopoietic cells (46). The COOH terminus of the related focal adhesion kinase FAK is similarly hydrophobic and mediates many signaling and cytoskeletal interactions (47, 48). Also of note is the finding of JNK kinase activation by VEGF or VRP in KS cells. Activation of JNK by OSM has been previously reported (39).

The finding that such a diverse group of cytokines is able to phosphorylate a single intracellular molecule which appears to link surface signals to the cytoskeleton and to pathways of transcriptional activation may help to explain the apparent redundancy of the growth promoting effects on KS cells of each of these specific cytokines. Further studies on the role of RAFTK in KS cells will aim to specifically inhibit its kinase function and evaluate the effects on downstream signaling pathways such as JNK.

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References

1. Dictor, M. 1988. Vascular remodeling in Kaposi's sarcoma and avian hemangiomatosis: relation to the vertebrate lymphatic system. *Lymphology.* 21: 53–60.

2. Dorfman, R.F. 1988. Kaposi's sarcoma: evidence supporting its origin from the lymphatic system. *Lymphology.* 21:45–52.

3. Chang, Y., E. Cesarman, M.S. Pessin, F. Lee, J. Culpepper, D.M. Knowles, and P.S. Moore. 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma [see comments]. *Science (Wash. DC).* 266:1865–1869.

4. Zhong, W., H. Wang, H.B. Herndier, and D. Ganem. 1996. Restricted expression of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) genes in Kaposi's sarcoma. *Proc. Natl. Acad. Sci. USA.* 93:6641–6646.

5. Huang, Y.Q., J.J. Li, W.G. Zhang, D. Feiner, and A.E. Friedman-Kien. 1996. Transcription of human herpesvirus-like agent (HHV-8) in Kaposi's sarcoma. *J. Clin. Invest.* 97:2803–2806.

6. Humphrey, R.W., T.R. O'Brien, F.M. Newcomb, H. Nishihara, K.M. Wyvill, G.A. Ramos, M.W. Saville, J.J. Goedert, S.E. Straus, and R. Yarchoan. 1996. Kaposi's sarcoma (KS)-associated herpesvirus-like DNA sequences in peripheral blood mononuclear cells: association with KS and persistence in patients receiving anti-herpesvirus drugs. *Blood.* 88:297–301.

7. Ensoli, B., G. Barillari, S.Z. Salahuddin, R.C. Gallo, and F. Wong-Staal. 1990. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature (Lond.).* 345:84–86.

8. Barillari, G., L. Buonaguro, V. Fiorelli, J. Hoffman, F. Michaels, R.C. Gallo, and B. Ensoli. 1992. Effects of cytokines from activated immune cells on vascular cell growth and HIV-1 gene expression. Implications for AIDS-Kaposi's sarcoma pathogenesis. *J. Immunol.* 149:3727–3734.

9. Buonaguro, L., G. Barillari, H.K. Chang, C.A. Bohan, V. Kao, R. Morgan, R.C. Gallo, and B. Ensoli. 1992. Effects of the human immunodeficiency virus type 1 Tat protein on the expression of inflammatory cytokines. *J. Virol.* 66:7159–7167.

10. Cai, J., P.S. Gill, R. Masood, P. Chandrasoma, B. Jung, R.E. Law, and S.F. Radka. 1994. Oncostatin-M is an autocrine growth factor in Kaposi's sarcoma. *Am. J. Pathol.* 145:74–79.

11. Ensoli, B., G. Barillari, and R.C. Gallo. 1992. Cytokines and growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Immunol. Rev.* 127:147–155.

12. Ensoli, B., S. Nakamura, S.Z. Salahuddin, P. Biberfeld, L. Larsson, B. Beaver, F. Wong-Staal, and R.C. Gallo. 1989. AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. *Science (Wash. DC).* 243:223–226.

13. Fiorelli, V., R. Gendelman, F. Samaniego, P.D. Markham, and B. Ensoli. 1995. Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. *J. Clin. Invest.* 95:1723–1734.

14. Huang, Y.Q., J.J. Li, K.S. Kim, A. Nicolaides, W.G. Zhang, J. Le, B.J. Poiesz, and A.E. Friedman-Kien. 1993. HIV-1 infection and modulation of cytokine and growth factor expression in Kaposi's sarcoma-derived cells in vitro. *AIDS (Phila.).* 7:317–322.

15. Ganem, D. 1995. AIDS. Viruses, cytokines and Kaposi's sarcoma. *Curr. Biol.* 5:469–471.

16. Miles, S.A. 1994. Pathogenesis of HIV-related Kaposi's sarcoma. *Curr. Opin. Oncol.* 6:497–502.

17. Ensoli, B., R. Gendelman, P. Markham, V. Fiorelli, S. Colombini, M. Raffeld, A. Cafaro, H.K. Chang, J.N. Brady, and R.C. Gallo. 1994. Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. *Nature (Lond.).* 371:674–680.

18. Miles, S.A., A.R. Rezai, J.F. Salazar-Gonzalez, M. Vander Meyden, R.H. Stevens, D.M. Logan, R.T. Mitsuyasu, T. Taga, T. Hirano, T. Kishimoto, and O. Martinez-Maza. 1990. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. *Proc. Natl. Acad. Sci. USA.* 87:4068–4072.

19. Nair, B.C., A.L. DeVico, S. Nakamura, T.D. Copeland, Y. Chen, A. Patel, T. O'Neil, S. Oroszlan, R.C. Gallo, and M.G. Sarngadharan. 1992. Identification of a major growth factor for AIDS-Kaposi's sarcoma cells as oncostatin M. *Science (Wash. DC).* 255:1430–1432.

20. Weindel, K., D. Marme, and H.A. Weich. 1992. AIDS-associated Kaposi's sarcoma cells in culture express vascular endothelial growth factor. *Biochem. Biophys. Res. Commun.* 183:1167–1174.

21. Yang, J., M.K. Hagan, and M.K. Offermann. 1994. Induction of IL-6 gene expression in Kaposi's sarcoma cells. *J. Immunol.* 152:943–955.

22. Brown, T.J., J.M. Rowe, J.W. Liu, and M. Shoyab. 1991. Regulation of IL-6 expression by oncostatin M. *J. Immunol.* 147:2175–2180.

23. Buonaguro, L., F.M. Buonaguro, G. Giraldo, and B. Ensoli. 1994. The human immunodeficiency virus type 1 Tat protein transactivates tumor necrosis factor beta gene expression through a TAR-like structure. *J. Virol.* 68:2677–2682.

24. Maier, J.A., M. Mariotti, A. Albini, P. Comi, M. Prat, P.M. Comoglio, and M.R. Soria. 1996. Over-expression of hepatocyte growth factor in human Kaposi's sarcoma. *Int. J. Cancer.* 65:168–172.

25. Sciacca, F.L., M. Sturzl, F. Bussolino, M. Sironi, H. Brandstetter, C. Zietz, D. Zhou, C. Matteucci, G. Peri, S. Sozzani, et al. 1994. Expression of adhesion molecules, platelet-activating factor, and chemokines by Kaposi's sarcoma cells. *J. Immunol.* 153:4816–4825.

26. Avraham, S., R. London, Y. Fu, S. Ota, D. Hiregowdara, J. Li, S. Jiang, L.M. Pasztor, R.A. White, J.E. Groopman, and H. Avraham. 1995. Identification and characterization of a novel related adhesion focal tyrosine kinase (RAFTK) from megakaryocytes and brain. *J. Biol. Chem.* 270:27742–27751.

27. Lev, S., H. Moreno, R. Martinez, P. Canoll, E. Peles, J.M. Musacchio, G.D. Plowman, B. Rudy, and J. Schlessinger. 1995. Protein tyrosine kinase Pyk2 involved in Ca^{2+}-induced regulation of ion channel and MAP kinase functions. *Nature (Lond.).* 376:737–745.

28. Sasaki, H., K. Nagura, M. Ishino, H. Tobioka, K. Kotani, and T. Sasaki. 1995. Cloning and characterization of cell adhesion kinase β, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily. *J. Biol. Chem.* 270: 21206–21219.

29. Li, J., H. Avraham, R.A. Rogers, S. Raja, and S. Avraham. 1996. Characterization of RAFTK, a novel focal adhesion kinase, and its integrin-dependent phosphorylation and activation in megakaryocytes. *Blood.* 88:417–428.

30. Kaipainen, A., J. Korhonen, T. Mustonen, V.W. van Hinsbergh, G.-H. Fang, D. Dumont, M. Breitman, and K. Alitalo. 1995. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc. Natl. Acad. Sci. USA.* 92:3566–3570.

31. Kaipainen, A., J. Korhonen, K. Pajusola, O. Aprelikova, M.G. Persico, B.I. Terman, and K. Alitalo. 1993. The related FLT4, FLT1, and KDR receptor tyrosine kinases show distinct expression patterns in human fetal endothelial cells. *J. Exp. Med.* 178:2077–2088.

32. Hewett, P.W., and J.C. Murray. 1996. Coexpression of flt-1, flt-4 and KDR in freshly isolated and cultured human endothelial cells. *Biochem. Biophys. Res. Commun.* 221:697–702.

33. Lee, J., A. Gray, J. Yuan, S-M. Luoh, H. Avraham, and W.I. Wood. 1996. Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4. *Proc. Natl. Acad. Sci. USA.* 93: 1988–1992.

34. Joukov, V., K. Pajusola, A. Kaipainen, D. Chilov, I. Lahtinen, E. Kukk, O. Saksela, N. Kalkkinen, and K. Alitalo. 1996. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the FLT4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO (Eur. Mol. Biol. Organ.) J.* 15: 290–298.

35. Tokiwa, G., I. Dikic, S. Lev, and J. Schlessinger. 1996. Activation of Pyk2 by stress signals and coupling with JNK signaling pathway. *Science (Wash. DC.).* 273:792–794.

36. Derijard, B., M. Hibi, I.-H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R.J. Davis. 1994. JNK-1: a protein-kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell.* 76:1025–1037.

37. Hibi, M., A. Lin, T. Smeal, A. Minden, and M. Karin. 1993. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* 7:2135–2148.

38. Kallunki, T., B. Su, I. Tsigelny, H.K. Sluss, B. Derijard, G. Moore, R. Davis, and M. Karin. 1994. JNK2 contains a specificity determining region responsible for efficient c-Jun binding and phosphorylation. *Genes Dev.* 8:2996–3007.

39. Faris, M., B. Ensoli, N. Stahl, G. Yancopoulis, A. Nguyen, S. Wang, and A.E. Nel. 1996. Differential activation of the extracellular signal-regulated kinase, Jun Kinase and Janus Kinase-Stat pathways by oncostatin M and basic fibroblast growth factor in AIDS-derived Kaposi's sarcoma cells. *AIDS (Phila.).* 10:369–378.

40. Masood, R., S.R. Husain, A. Rahman, and P. Gill. 1993. Potentiation of cytotoxicity of Kaposi's sarcoma related to immunodeficiency syndrome (AIDS) by liposome-encapsulated doxorubicin. *AIDS Res. Hum. Retroviruses.* 9:741–746.

41. Masood, R., Y. Lunardi-Iskandar, L.F. Jean, J.R. Murphy, C. Waters, R.C. Gallo, and P. Gill. 1994. Inhibition of AIDS-associated Kaposi's sarcoma cell growth by DAB389-interleukin 6. *AIDS Res. Hum. Retroviruses.* 10:969–975.

42. Guo, W., T. Antakly, M. Cadotte, Z. Kachra, L. Kunkel, R. Masood, and P.S. Gill. 1996. Expression and cytokine regulation of glucocorticoid receptors in Kaposi's sarcoma. *Am. J. Pathol.* 148:1999–2007.

43. Brown, L.F., K. Tognazzi, H.F. Dvorak, and T.J. Harrist. 1996. Strong expression of kinase insert domain-containing receptor, a vascular permeability factor/vascular endothelial growth factor receptor in AIDS-associated Kaposi's sarcoma and cutaneous angiosarcoma. *Am. J. Pathol.* 148:1065–1074.

44. Amaral, M.C., S. Miles, G. Kumar, and A.E. Nel. 1993. Oncostatin-M stimulates tyrosine protein phosphorylation in parallel with the activation of $p42^{MAPK}$/ERK-2 in Kaposi's cells. Evidence that this pathway is important in Kaposi cell growth. *J. Clin. Invest.* 92:848–857.

45. Angel, P., K. Hattori, T. Smeal, and M. Karin. 1988. The *jun* proto-oncogene is positively autoregulated by its product, Jun/AP-1. *Cell.* 55:875–885.

46. Salgia, R., S. Avraham, E. Pisick, J.-L. Li, S. Raja, E.A. Greenfield, M. Sattler, H. Avraham, and J.D. Griffin. 1996. The related adhesion focal tyrosine kinase forms a complex with paxillin in hematopoietic cells. *J. Biol. Chem.* 271: 31222–31226.

47. Clark, E.A., and J.S. Brugge. 1995. Integrins and signal transduction pathways. *Science (Wash. DC).* 268:233–239.

48. Bellis, S.L., J.T. Miller, and C.E. Turner. 1995. Characterization of tyrosine phosphorylation of paxillin *in vitro* by focal adhesion kinase. *J. Biol. Chem.* 270:17437–17441.

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Activation of Pyk2 **by stress signals and coupling with JNK signaling pathway.**

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The c-Jun amino-terminal kinase (JNK) is activated by various heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors, inflammatory cytokines, and stress signals. Yet, upstream mediators that link extracellular signals with the JNK signaling pathway are currently unknown. The tyrosine kinase **Pyk2** was activated by tumor necrosis factor alpha, by ultraviolet irradiation, and by changes in osmolarity. Overexpression of **Pyk2** led to activation of JNK, and a dominant-negative mutant of **Pyk2** interfered with ultraviolet light- or osmotic shock-induced activation of JNK. **Pyk2** represents a cell type-specific, stress-sensitive mediator of the JNK signaling pathway.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: *Ca(2+)-Calmodulin Dependent Protein Kinase--metabolism--ME; *Mitogen-Activated Protein Kinases; *Protein-Tyrosine Kinase--metabolism --ME; *Signal Transduction; Animals; Anisomycin--pharmacology--PD; Egtazic Acid--pharmacology--PD; Enzyme Activation; GTP Phosphohydrolases --metabolism--ME; GTP-Binding Proteins--metabolism--ME; HL-60 Cells; Osmolar Concentration; PC12 Cells; Phosphorylation; Rats; Sorbitol --pharmacology--PD; Transfection; Tumor Necrosis Factor--pharmacology--PD; Ultraviolet Rays

CAS Registry No.: 0 (Tumor Necrosis Factor); 22862-76-6 (Anisomycin); 50-70-4 (Sorbitol); 67-42-5 (Egtazic Acid)

Enzyme No.: EC 2.7.1.- (protein tyrosine kinase **PYK2**); EC 2.7.1.112 (Protein-Tyrosine Kinase); EC 2.7.1.123 (Ca(2+)-Calmodulin Dependent Protein Kinase); EC 2.7.1.37 (Mitogen-Activated Protein Kinases); EC 2.7.10.- (c-Jun amino-terminal kinase); EC 3.6.1.- (GTP Phosphohydrolases); EC 3.6.1.- (GTP-Binding Proteins)

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Activation of Pyk2 by Stress Signals and Coupling with JNK Signaling Pathway

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The c-Jun amino-terminal kinase (JNK) is activated by various heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors, inflammatory cytokines, and stress signals. Yet, upstream mediators that link extracellular signals with the JNK signaling pathway are currently unknown. The tyrosine kinase Pyk2 was activated by tumor necrosis factor α, by ultraviolet irradiation, and by changes in osmolarity. Overexpression of Pyk2 led to activation of JNK, and a dominant-negative mutant of Pyk2 interfered with ultraviolet light– or osmotic shock–induced activation of JNK. Pyk2 represents a cell type–specific, stress-sensitive mediator of the JNK signaling pathway.

Various cellular stimuli that control cell growth and differentiation use small guanine triphosphatases (GTPases) and kinase cascades to transmit signals from the cell surface to the nucleus. Activation of the small GTPase Ras is critical for stimulation of a kinase cascade composed of Raf, mitogen-activated protein kinase kinase (MAPKK), and mitogen-activated protein kinase (MAPK) (*1*). A related signaling pathway is activated by G protein–coupled receptors, inflammatory cytokines, and stress signals such as ultraviolet (UV) light and osmotic shock (*2*–*4*). Key elements of this pathway include the Rho-like GTPases, CDC42Hs and Rac (*3*, *5*, *6*), and a kinase cascade that phosphorylates and activates JNK, which is also known as the stress-activated protein kinase (SAPK) (*2*). Activated JNK phosphorylates c-Jun (*7*, *8*), leading to stimulation of AP-1 transcriptional activity (*9*).

We recently discovered a protein tyrosine kinase, termed Pyk2, that is related to the focal adhesion kinase (*10*) and is activated by various extracellular signals that increase intracellular calcium concentrations (*11*). Pyk2 can tyrosine phosphorylate and modulate the action of ion channels as well as feed into and activate the Ras-MAPK signaling pathway (*11*). Pyk2 appears to function as an intermediate that links various calcium signals with both short- and long-term responses in neuronal cells.

We examined whether Pyk2 could be activated by tumor necrosis factor–α (TNF-α) and stress signals and whether it could function in the control of the JNK signaling pathway activated by these stimuli. We tested the effect of TNF-α on the status of Pyk2 phosphorylation in human promyelocytic leukemia (HL-60) cells and in rat pheochromocytoma (PC-12) cells (Fig. 1, A and B). Stimulation with TNF-α led to enhanced tyrosine phosphorylation of Pyk2 in HL-60 and PC-12 cells (Fig. 1).

We examined the status of Pyk2 phosphorylation in response to three other activators of JNK signaling. PC-12 cells were subjected to UV irradiation, sorbitol treatment to increase extracellular osmolarity, and anisomycin treatment to inhibit protein synthesis (*3*, *4*, *12*). Ultraviolet irradiation and sorbitol treatment of PC-12 cells induced tyrosine phosphorylation of Pyk2, whereas anisomycin treatment did not (Fig. 1B). Hence, not all stress signals stimulate activation of Pyk2.

Agonists such as carbachol stimulate Pyk2 activation by stimulation of extracellular calcium influx (*11*). To examine the effect of extracellular calcium influx on TNF-α–, sorbitol-, or UV-induced activation of Pyk2, we treated PC-12 cells with TNF-α, sorbitol, or exposed the cells to UV light in the presence or absence of the calcium chelator EGTA (3 mM) (*12*). JNK activity was also examined in the same lysates (*7*, *13*, *14*). As a control, the effect of EGTA on anisomycin activation of JNK was examined. Both UV light– and sorbitol-induced activation of Pyk2 were substantially reduced in cells that were stimulated in the presence of EGTA, whereas TNF-α–induced activation of Pyk2 was not affected by the presence of EGTA (Fig. 1B). This experiment demonstrates that UV light– or sorbitol-induced activation of Pyk2 can be mediated in part by the increase in intracellular calcium concentration that is induced by these stimuli.

In the presence of EGTA, JNK activity from UV-induced cells was reduced by approximately 20%, whereas JNK activity from sorbitol-induced cells was reduced by approximately 35% (Fig. 1C). In the presence of EGTA, however, stimulation of Pyk2 and JNK activity by TNF-α was not affected by EGTA, demonstrating that activation of Pyk2 and JNK is not mediated by TNF-α–induced influx of extracellular calcium (Fig. 1, B and C). Similarly, EGTA had no effect on anisomycin-induced activation of JNK in these cells.

Both UV- and sorbitol-induced JNK activation are largely calcium independent. It is likely that stress signals activate the JNK signaling pathway by means of several upstream mediators; Pyk2 may function as one but not the only upstream mediator of UV- or sorbitol-induced JNK activation. This hypothesis is consistent with the smaller effect of EGTA on UV- and sorbitol-in-

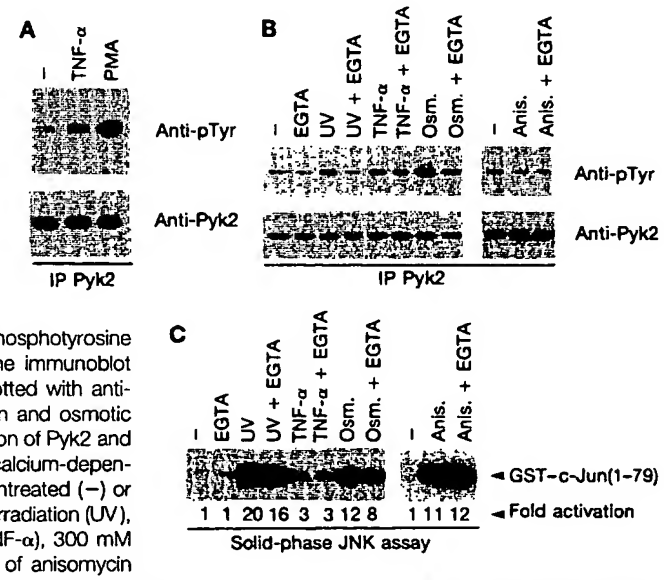

Fig. 1. Phosphorylation of Pyk2 in cells exposed to TNF-α and stress stimuli. (**A**) HL-60 cells were left unstimulated (–) or stimulated with human recombinant tumor necrosis factor (30 ng/ml) (TNF-α) or 1 μM phorbol 12-myristate 13-acetate (PMA) as a control (*11*, *12*). The cells were lysed, Pyk2 was immunoprecipitated (IP) with anti-Pyk2, and analyzed by immunoblotting with anti-phosphotyrosine (anti-pTyr) (*11*, *13*). The same immunoblot was stripped and immunoblotted with anti-Pyk2. (**B**) Ultraviolet irradiation and osmotic shock–induced phosphorylation of Pyk2 and JNK activation are partially calcium-dependent. PC-12 cells were left untreated (–) or exposed to 80 J/m² of UV-C irradiation (UV), murine TNF-α (50 ng/ml) (TNF-α), 300 mM sorbitol (Osm.), or 20 μg/ml of anisomycin (Anis.) in the presence or absence of 3 mM EGTA (*12*). Tyrosine phosphorylation of Pyk2 was analyzed as described in (A). (**C**) Solid-phase JNK assays were done with 2 μg of GST–c-Jun(1–79) immobilized on glutathione-agarose beads as the substrate (*7*, *14*). The experiment was repeated three times with similar results. Fold activation of the experiment is indicated. Minus (–) indicates unstimulated cells.

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duced activation of JNK as compared to the effects of EGTA on stress signal–induced activation of Pyk2 in the same cells.

Overexpression of Pyk2 in human embryonic kidney 293T cells led to phosphorylation of the glutathione-S-transferase (GST)–c-Jun(1–79) fusion protein (*7*, *14*), revealing clear Pyk2-dependent activation of JNK in these cells (Fig. 2). The effect was dependent on the level of expression of Pyk2. We also analyzed the activity of epitope-tagged (*15*) JNK that was co-expressed with a Pyk2 expression vector (*16*). Overexpression of Pyk2 caused activation of co-expressed hemagglutinin (HA)-tagged JNK (*17*). JNK activity was monitored from cells that were incubated with conditioned medium taken from cells transfected with a Pyk2 expression vector. JNK was not activated in these cells, which ruled out activation of JNK by a Pyk2-induced autocrine loop (Fig. 2, B and C).

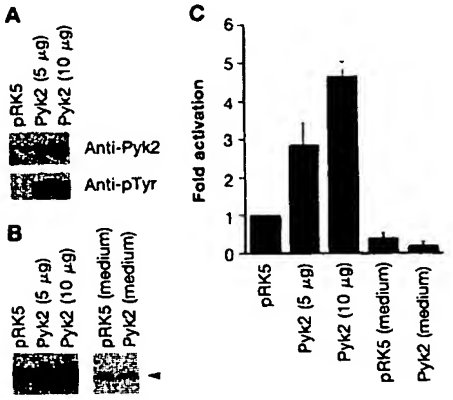

Fig. 2. Activation of JNK by overexpression of Pyk2. (**A**) 293T cells were transiently transfected with expression vector alone (pRK5) or with 5 or 10 μg of a Pyk2 expression vector (*16*). Pyk2 was immunoprecipitated from cell lysates after 48 hours and analyzed as described (*11*, *13*). (**B**) Solid-phase JNK assays were performed (*7*, *14*) from lysates in (A). Also, medium was taken from cells transfected with pRK5 or Pyk2 (10 μg) after 48 hours and incubated for 20 min with untransfected 293T cells. These cells were lysed and assayed for JNK activation. The arrowhead in (B) indicates GST–c-Jun(1–79). The experiment was repeated four times and (**C**) shows fold activation in JNK assays (± SD).

To test whether Pyk2 is required as an upstream regulator of the JNK signaling pathway, we used a catalytically inactive mutant of Pyk2 (PKM) that acts as a dominant-negative inhibitor of wild-type Pyk2 in PC-12 cells (*11*). PC-12 cells were transiently transfected with a PKM expression vector (*16*). After 48 hours, cells were irradiated with UV light or treated with sorbitol, lysed, and analyzed for the activation of JNK (*7*, *13*, *14*). Expression of PKM inhibited both UV- and sorbitol-induced JNK activation (Fig. 3A). However, overexpression of PKM did not affect UV- and sorbitol-induced JNK activation in 293T or COS cells, which do not express Pyk2 (Fig. 3B).

These results suggest that Pyk2 can act as a cell type–specific and stress-sensitive mediator of the JNK signaling pathway. Recent studies demonstrated that dominant-negative mutants of the small GTPases, CDC42Hs and Rac1, can block JNK activation induced by stress responses, inflammatory cytokines, or tyrosine kinases (*3*, *5*, *6*, *18*). We examined whether Pyk2 acts upstream of the small GTPases, CDC42Hs, Rac1, RhoA, and Ras. Expression of dominant-negative mutants of CDC42Hs(N17), Rac1(N17), or Ras(N17) efficiently blocked Pyk2-induced activation of JNK. Expression of a dominant-negative mutant of RhoA(N19), however, did not influence Pyk2-induced activation of JNK (*17*).

The protein tyrosine kinase Pyk2 is activated by the inflammatory cytokine TNF-α, by UV irradiation, and by changes in osmolarity and can function as an upstream mediator of the JNK signaling pathway. The mechanism by which Pyk2 activates the JNK signaling pathway is currently unknown. Pyk2 can activate the Ras-MAPK signaling pathway by both direct and indirect recruitment of the adapter proteins Grb2 and Shc (*11*). These two adapter proteins may also play a role in the activation of JNK because a dominant-negative mutant of Ras conferred partial inhibition of tyrosine kinase–induced activation of JNK (*19*) as well as partial inhibition of Pyk2-induced activation of JNK in 293T cells. The molecular link or links between Pyk2 and CDC42Hs (or Rac1) are also not known. The primary structure of Pyk2 contains putative binding sites for phosphatidylinositol (PI) 3-kinase (*11*); PI 3-kinase may serve as an intermediate between protein tyrosine kinases and Rho-like GTPases (*20*). Pyk2 can be activated by a variety of G protein–coupled receptors that are able to activate the MAPK signaling pathway (*11*, *21*). Hence, Pyk2 has the potential to activate the MAPK signaling pathway, the JNK signaling pathway, or both signaling pathways in the context of different cells and in response to different extracellular stimuli.

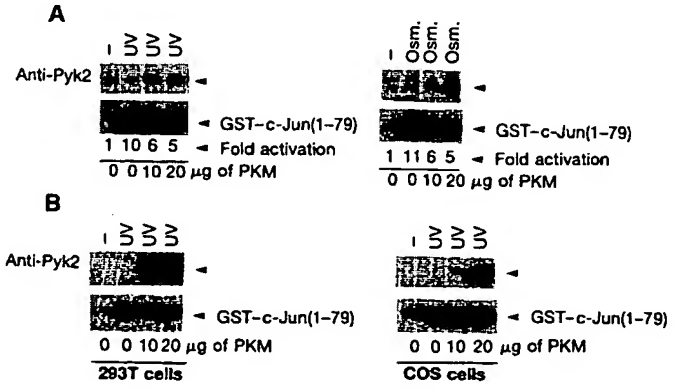

Fig. 3. Effects of dominant-negative PKM on JNK activation. (**A**) PC-12 cells were transiently transfected with expression vector alone (pRK5) or various amounts of a dominant-negative Pyk2 mutant expression vector (PKM) (*11*, *16*). After 48 hours, transfected cells were either exposed to 80 J/m² of UV-C irradiation (left) or osmotically shocked in 300 mM sorbitol containing medium and lysed (right) (*12*, *13*). Total cell lysates were used for immunoblot analysis with anti-Pyk2 to monitor PKM expression (upper panel) (*11*). The top arrowhead indicates the total contribution of endogenous Pyk2 and PKM identified by anti-Pyk2. Solid-phase JNK assays were done as described (*7*, *14*). The experiment was repeated three times with similar results. Fold increases for the experiment are indicated. (**B**) 293T cells (left) and COS cells (right) were transiently transfected with various amounts of PKM or pRK5 (*16*). After 48 hours, transfected cells were exposed to UV-C irradiation and treated as in (A). Total cell lysates were used for PKM immunoblot analysis (top arrowhead), and solid-phase JNK assays were done as described (*7*, *14*).

REFERENCES AND NOTES

1. J. Schlessinger, *Trends Biochem. Sci.* **18**, 273 (1993); C. J. Marshall, *Cell* **80**, 179 (1995); C. S. Hill and R. Treisman, *ibid.*, p. 187.
2. B. Derijard *et al.*, *Cell* **76**, 1025 (1994); J. M. Kyriakis *et al.*, *Nature* **369**, 156 (1994).
3. O. A. Coso *et al.*, *Cell* **81**, 1137 (1995).
4. Z. Galcheva-Gargova, B. Derijard, I.-H. Wu, R. J. Davis, *Science* **265**, 806 (1994).
5. A. Minden, A. Lin, F.-X. Claret, A. Abo, M. Karin, *Cell* **81**, 1147 (1995).
6. C. S. Hill, J. Wynne, R. Treisman, *ibid.*, p. 1159.
7. M. Hibi, A. Lin, T. Smeal, A. Minden, M. Karin, *Genes Dev.* **7**, 2135 (1993); A. Minden *et al.*, *Mol. Cell. Biol.* **14**, 6683 (1994).
8. B. Su *et al.*, *Cell* **77**, 727 (1994).
9. P. Angel, K. Hattori, T. Smeal, M. Karin, *ibid.* **55**, 875 (1988).
10. M. D. Schaller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5192 (1992); H. Sasaki *et al.*, *J. Biol. Chem.* **270**, 21206 (1995); S. Avraham *et al.*, *ibid.*, p. 27742.
11. S. Lev *et al.*, *Nature* **376**, 737 (1995).
12. PC-12, COS, and 293T cells were grown as described (*5*, *11*). For UV irradiation (80 J/m² UV-C), osmotic-shock activation (with 300 mM sorbitol) as well as treatments with TNF-α (50 ng/ml), PMA (1 μM), or with anisomycin (20 μg/ml) were performed as described (*4*, *7*, *11*). Untreated cells (−) had the medium removed and then immediately replaced. When appropriate, cells were also treated as described in the presence of 3 mM EGTA (*11*) [F. M. Mitchell, M. Russell, G. L. Johnson, *Biochem. J.* **309**, 381 (1995)]. HL-60 cells were grown to 1 × 10⁷ to 2 × 10⁷ cells per milliliter in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cells were collected by centrifugation and treated with human recombinant

TNF-α (30 ng/ml) or with PMA (1 μM).

13. Cell lysis, Pyk2 immunoprecipitations, and immunoblotting were carried out as previously described (*11*). Polyclonal antibodies to amino acids 684 to 1009 of human Pyk2 cDNA (anti-Pyk2) expressed as a GST fusion protein in pGEX-2T (Pharmacia Biotech) and phosphotyrosine antibodies were used. Equal amounts of total cell lysate (500 to 700 μg) as determined by Bradford assay (Bio-Rad) were subjected to immunoprecipitation.
14. GST–c-Jun(1–79) fusion proteins were produced and bound to agarose beads as described (*7*). Bound protein (2 μg) was used in solid-phase JNK assays. Lysates were incubated with the GST fusion protein, and after JNK proteins were bound, the beads were washed extensively, [γ-^{32}P]adenosine triphosphate was added, and the phosphorylation of the probe was analyzed by SDS–polyacrylamide gel electrophoresis and autoradiography. JNK activity was determined by quantitation of the amount of GST–c-Jun(1–79) phosphorylation with a Molecular Dynamics PhosphorImager and ImageQuant software (Molecular Dynamics).
15. J. Field *et al.*, *Mol. Cell. Biol.* **8**, 2159 (1988).
16. 293T cells grown on 10-cm plates were transiently transfected [lipofectamine reagent (Gibco BRL)] with various amounts (5, 10, or 15 μg) of expression vector alone (pRK5); 5 or 10 μg of Pyk2 expression vector together with various amounts of pRK5 (5 or 10 μg); or 5 μg of Pyk2 together with 10 or 20 μg of a dominant-negative mutant of Pyk2 (PKM). COS and PC-12 cells on 10-cm plates were transiently transfected with 10 or 20 μg of pRK5 and 10 or 20 μg of PKM. After 48 hours, the cells were treated as described (*11*, *13*).
17. G. Tokiwa, I. Dikic, S. Lev, J. Schlessinger, unpublished results.
18. R. Kozma, A. Ahmed, A. Best, L. Lim, *Mol. Cell. Biol.* **15**, 1942 (1995).
19. A. Minden *et al.*, *Science* **266**, 1719 (1994).
20. P. T. Hawkins *et al.*, *Curr. Biol.* **5**, 393 (1995).
21. I. Dikic, G. Tokiwa, S. Lev, S. A. Courtneidge, J. Schlessinger, unpublished results.

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Cooperative DNA Binding and Sequence-Selective Recognition Conferred by the STAT Amino-Terminal Domain

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STAT proteins (signal transducers and activators of transcription) activate distinct target genes despite having similar DNA binding preferences. The transcriptional specificity of STAT proteins was investigated on natural STAT binding sites near the interferon-gamma gene. These sites are arranged in multiple copies and required cooperative interactions for STAT binding. The conserved amino-terminal domain of STAT proteins was required for cooperative DNA binding, although this domain was not essential for dimerization or binding to a single site. Cooperative binding interactions enabled the STAT proteins to recognize variations of the consensus site. These sites can be specific for the different STAT proteins and may function to direct selective transcriptional activation.

Cytokines activate intracellular signaling pathways during immune and inflammatory responses. One of the signal-transduction pathways activated by these ligands involves the STAT family of proteins (*1*). STAT proteins are selectively recruited to various cytokine receptors by virtue of their peptide-binding specificities mediated by their SRC homology 2 (SH2) domains (*2*). Thus, part of the specificity of STAT activation relies on selective recognition of phosphotyrosine-containing peptides of cytokine receptors. After they are tyrosine phosphorylated, the STAT proteins dimerize and migrate to the nucleus where they bind to specific DNA sequences and elicit various programs of gene activation. The mechanisms that determine STAT specificity in transcriptional regulation are unclear because most of the STAT proteins have similar DNA binding preferences (*3*, *4*).

The STAT proteins contain a DNA binding domain that is located in the center of the protein, approximately from residues 350 to 500 (*3*). Other functional domains include the SH2 domain and a phosphotyrosine-containing region near the COOH-terminus that participate in dimerization. The transcription-activation domains are located at the COOH-terminal ends of the molecules (*5*). The NH_2-terminal region is also conserved among the STAT family. This region is required for phosphorylation of STAT2 in response to interferon-α (IFN-α) and may be required for receptor binding (*6*). It is not known whether this region plays a similar role in other STAT proteins or whether the NH_2-terminal domain takes part in the nuclear function of these proteins as transcription factors.

We investigated the mechanisms of STAT regulation in response to interleukin-12 (IL-12), a key cytokine that regulates cell-mediated immunity (*7*). Treatment of cells with IL-12 results in tyrosine phosphorylation and activation of STAT4 (*8*). A full-length STAT4 cDNA was cloned and expressed in baculovirus-infected insect cells (*9*). The DNA binding preference of STAT4 was determined by the random binding site selection method (*10*), and the optimal sequence for STAT4 was TTCCGGGAA (*11*). The sequence selected by STAT4 is identical to that of optimal sites for STAT1 and STAT3 (*3*). STAT5 also has a similar binding preference (*12*).

We also searched for natural STAT4 sites near the IFN-γ gene. IFN-γ is activated in response to IL-12 in T cells and natural killer cells, and many of the physiological effects of IL-12 appear to be mediated by IFN-γ (*7*). IL-12–mediated activation of IFN-γ expression is eliminated in STAT4-deficient mice (*13*). The human IFN-γ gene is organized on four exons (*14*). A 9-kb region spanning the gene contains the necessary cis-regulatory sequences for the correct T cell–specific and inducible transcription of IFN-γ (*15*). The first intron contains a deoxyribonuclease I (DNase I)–hypersensitive site, ~0.5 to 1 kb downstream of the transcription start site (*16*). Using DNase I footprinting (*17*), we detected binding sites for STATs 1, 4, 5, and 6 in the first intron (Fig. 1A). On the basis of our binding site selection results, we expected that STATs 1, 4, and 5 might have the same site preferences. Instead, we observed that each of the STAT proteins bound to a distinct pattern of adjacent sites in the first intron. The protected regions contain multiple sites that are variations of STAT consensus binding sequence (Fig. 1B).

The STAT4 footprint spans ~30 base pairs (bp) of DNA and contains two adjacent binding sites, designated 2 and 3 (Fig. 1B). Each binding site is a variant of the consensus sequence, and the two sites are spaced by 10 bp (*18*). The STAT4 binding sites vary from the optimum sequence in two ways. In both cases the sites are imperfect palindromes, with TTC in one half-site and TTA in the other half-site, and the central three nucleotides are different from those in the optimal site, which has CGG in the middle.

STAT1 also bound to site 3. STAT1 protected a large region that extends downstream and appears to contain at least four additional sites, 4, 5, 6, and 7. The other STAT proteins tested did not bind well to sites 4 through 7 (Fig. 1A). STAT5 bound to sites 2 and 3 and also to a third site that was immediately upstream, site 1. STATs 1 and 4 did not bind to site 1, and STAT6 bound only to site 1. The results indicate that STAT proteins can have selective

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Signal transduction. Team blue sees red.

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Molecules in focus
Paxillin

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Abstract

Paxillin is a 68 kDa cytoplasmic protein that localizes to discrete sites of cell attachment to the extracellular matrix called focal adhesions. It is a multi-domain adapter protein capable of interacting with several structural and signaling proteins including vinculin, FAK, PYK2, Src and Crk. Phosphorylation of paxillin in response to integrin-mediated cell adhesion and growth factor stimulation regulates some of these interactions. Thus, paxillin functions as a scaffold for the recruitment of molecules into a signal transduction complex that is closely apposed to the plasma membrane. This is likely to facilitate the efficient processing of external stimuli that modulate important cellular events including cell adhesion, cell motility and growth control. Since paxillin interacts with several proteins known to cause cell transformation, the binding sites for these proteins on paxillin represent potential targets for therapeutic agents.

1. Introduction

Cell adhesion and cell migration are essential components of many biological processes including embryonic development, wound repair and the transition of non-malignant tumors to a metastatic phenotype. These processes are regulated in part by signals received by cells through contact with the extracellular matrix, in combination with exposure to soluble growth factors [1, 2]. Normal cells are absolutely dependent on adhesion and growth factors to generate the signals necessary for proliferation, while transformed cells are able to by-pass these growth control checkpoints.

Specialized structures referred to as focal adhesions mediate the physical linkage between the extracellular matrix and the actin-based cytoskeleton within the cell. Focal adhesions consist of hetero-dimeric transmembrane matrix receptors known as integrins that also interact via their cytoplasmic tails with a complex of intracellular structural proteins and the actin cytoskeleton. Additionally, there are multiple regulatory proteins, such as kinases and phosphatases concentrated at these sites that serve to efficiently transduce the extracellular signals leading to modulation of adhesion and gene expression [1–3]. The 68 kDa focal adhesion protein paxillin [4] is a multi-domain molecule that contains binding sites for many of these structural and regulatory molecules [5]. Some of these binding sites, including the SH2-binding motifs, are available for interaction with their ligand only when they are

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phosphorylated. These features suggest an important role for paxillin in recruiting common signal cascade components into close proximity with each other thereby permitting efficient transduction of signals from one component to the next. Additionally, paxillin may be a signal transduction crossroads for integration of growth factor and adhesion activation signals.

2. Structure

Paxillin was originally purified from smooth muscle tissue (chicken gizzards) and used in blot overlay assays to demonstrate an interaction with another focal adhesion protein, vinculin [4]. Cloning of the chicken paxillin cDNA [5] permitted the identification of multiple structural features (Fig. 1). The 68 kDa protein (559 amino acids) can be divided superficially into two distinct structural domains. The first comprises the amino-terminal 325 amino acids and contains binding sites for vinculin, and the non-receptor tyrosine kinases focal adhesion kinase (FAK) and the FAK-related kinase, PYK2 [6]. Careful analysis of these binding sites on paxillin revealed that they each contain a novel 8 amino acid repeating sequence. These have been named paxillin LD motifs because of the invariant leucine–aspartate pairing of amino acids that begin the repeat [6]. There are, in fact, four well conserved LD motifs and one with less homology, encoded within the amino-terminus of paxillin. These motifs, in turn, interact with common paxillin binding subdomains (PBS) within vinculin and FAK [7]. This suggests that LD repeats represent a novel protein binding interface. The more recent demonstration of an interaction between the papillomavirus oncoprotein, E6, and the LD motifs of paxillin [8] provides further support for this hypothesis. The amino terminal half of paxillin also contains proline-rich domains that interact with the SH3 domains of Src and Crk family members [9].

The carboxyl-terminus (amino acids 326–559) of paxillin is composed exclusively of four LIM domains. These are double zinc finger motifs, each of approximately 50 amino acids. LIM domains are found in a wide variety of proteins including transcription factors as well as cytoskeleton-associated molecules. In general, they serve as another protein–protein binding module. In the case of paxillin, the third LIM domain is absolutely essential for targeting the protein to focal adhesions [6]. The molecule that recruits paxillin to focal adhesions via this domain remains to be determined.

Importantly, paxillin is also a phosphoprotein. The phosphorylation status of the protein is modulated by cell adhesion to extracellular matrix and following exposure of cells to growth factors [10, 11]. Some of these modifications lead to the generation of yet more protein binding modules. For example, phosphorylation sites for the tyrosine kinases FAK and/or Src have been identified in the amino terminus (Y31 and 118) and these serve as SH2 domain recognition sites for the adaptor protein Crk [12, 13]. Subsequent binding of Crk to the Ras guanine nucleotide exchange factor, C3G may provide a mechanism by which paxillin could contribute to Ras activation of the mitogen-activated protein kinase (MAPK) cascade. Both v-Src and v-Crk cause transformation of fibroblasts. Thus, phosphorylation in response to the activity of these oncogenes may perturb a role for paxillin in normal growth control.

Paxillin is also phosphorylated on serine residues 188 and 190 following adhesion to fibronectin by an unidentified kinase. The function of these modifications is currently unknown [14]. Specific sites of serine and threonine phosphorylation have recently been identified on LIM2 and LIM3 of paxillin. Phosphorylation of these sites is important for the efficient targeting of paxillin to focal adhesions and may also regulate cell adhesion to the extracellular matrix [15]. Paxillin also contains multiple consensus sites for serine/threonine kinases such as protein kinases A and C. Clearly, the action of phosphatases is also likely to represent an important mechanism for the regulation of paxillin function. In this regard tyrosine dephosphorylation of paxillin is observed following insulin and cAMP stimulation of cells and is associated with distinct changes in the cytoskeleton and cell growth rates.

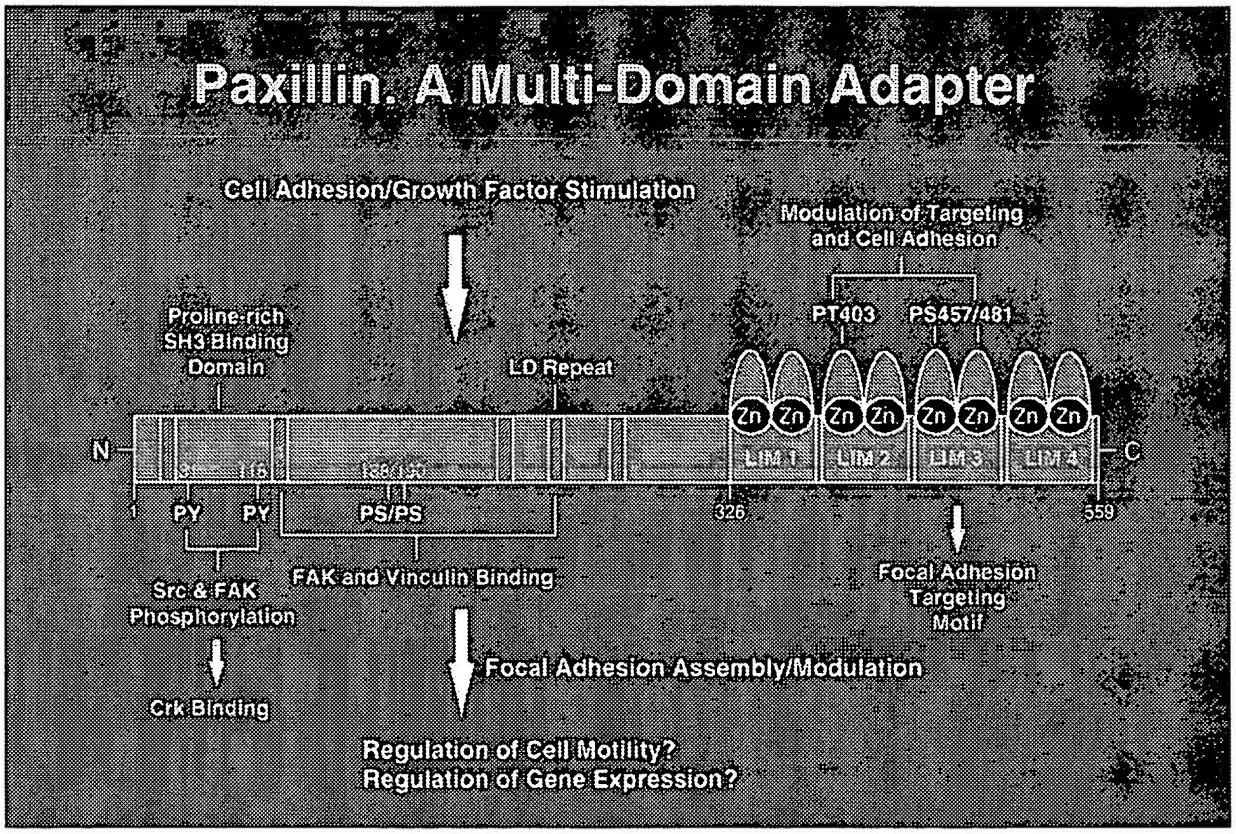

Fig. 1. Structural and functional domains of paxillin. Paxillin can be subdivided into several domains that are each involved in mediating protein–protein interactions. Novel paxillin LD motifs in the amino-terminus support the binding of vinculin, FAK and the E6 onco-protein. Proline-rich regions mediate associations with the SH3 domains of Src and Crk. Phosphorylation of tyrosine 31 and 118 by FAK and/or Src generates binding sites for the SH2 domain of Crk. Serine residues 188/190 of paxillin are phosphorylated during cell adhesion to fibronectin, although the function of these modifications is unclear at present. The carboxy-terminus contains four LIM domains. LIM 3 is essential for targeting paxillin to focal adhesions. The phosphorylation of LIM 2 and 3 by associated serine/threonine kinases is stimulated by adhesion and soluble growth factors and regulates the localization of paxillin to focal adhesions. Functions for the other LIM domains have not been assigned. By utilizing these multiple binding domains to recruit appropriate effector molecules, paxillin is positioned to efficiently transduce growth factor- and adhesion-derived signals into cellular responses such as migration, proliferation and gene expression.

3. Synthesis and degradation

Paxillin is most abundant in smooth muscle tissue [10], such as the walls of the vasculature and the uterus. This is perhaps not surprising since the attachment of actin filaments to the extracellular matrix in smooth muscle tissue occurs via specialized structures called dense plaques that are analogous in protein composition to focal adhesions. This organization permits the efficient transduction across the plasma membrane of force generated during muscle contraction.

Paxillin is present in lesser amounts in most other tissues with very low levels in the brain. Paxillin expression is not confined to organized tissues. Circulating leukocytes, including lymphocytes and macrophages express the protein at quite high levels but, unlike many other focal adhesion proteins, paxillin is absent from platelets. Currently, there is little information regarding the synthesis and degradation of paxillin.

Metabolic labeling studies using cultured fibroblasts indicate that the protein is quite stable when compared to other focal adhesion proteins such as talin and vinculin (Turner, unpublished observations). However, in some cell types paxillin may be degraded during mitosis [16].

4. Biological function

One of the principal functions of paxillin is as a multi-domain adapter molecule [5]. Such molecules are important for efficient signal transduction because they recruit multiple structural and signaling proteins to specific locations within the cell. In this context, paxillin recruits molecules to the plasma membrane for efficient processing of integrin- and growth factor-mediated signals derived from the extracellular environment. Evidence for such a role comes primarily from the study of cultured cell systems such as vascular smooth muscle cells. Stimulation of these cells with angiotensin II and thrombin for example, leads to paxillin phosphorylation and cytoskeletal reorganization [10]. These changes likely regulate pathways leading to smooth muscle cell proliferation and migration associated with hypertension and atherosclerosis in vivo. Interestingly, the phosphorylation state of paxillin is also altered dramatically during embryonic development [10], a process that requires considerable modulation of cell–matrix and cell–cell interactions. Determining the precise role of paxillin phosphorylation/dephosphorylation in the regulation of cell motility, cell adhesion and gene expression represent important areas of current study.

It is noteworthy that another molecule with extensive homology to paxillin, called Hic-5 has recently been described. Importantly, this protein is co-expressed with paxillin and shares several features including focal adhesion localization, LD motifs that support vinculin, FAK and PYK2 binding and four LIM domains [6, 17]. However, there are also important differences. Hic-5 lacks the principal tyrosine phosphorylation and SH3-binding sites of paxillin and, rather than being involved in growth stimulatory pathways as is the case with paxillin, Hic-5 expression is up-regulated in senescent cells. This balance of complementing and distinct features suggests that paxillin and Hic-5 may compete for the binding of important regulatory molecules and by so doing modulate the effect of these proteins on down-stream signaling pathways.

5. Possible medical and industrial applications

The human paxillin gene is located on chromosome 12q24 [18]. It has not, as yet, been directly associated with any genetic disorder. However, the gene locus involved in causing Dariers disease, a keratinocyte adhesion disease, as well as the locus for an autosomal dominant cerebellar ataxia have been mapped close to the paxillin gene. As mentioned above, paxillin is most abundant in smooth muscle and is an intermediary in growth factor signaling in these cells, suggesting an important role in vascular hemostasis. The ability of paxillin to interact with several oncoproteins including v-src, v-crk and the papillomavirus E6 protein indicates that such associations might be important in regulating the activity of these transforming proteins. As such, the generation of small synthetic peptides with the potential to perturb these interactions with paxillin might prove effective in restoring the normal growth characteristics of the infected cells.

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References

[1] J.A. Clark, J.S. Brugge, Integrins and signal transduction pathways, Science (Wash. DC) 268 (1995) 233–239.
[2] S.W. Craig, R.P. Johnson, Assembly of focal adhesions: progress, paradigms and portents, Curr. Opin. Cell Biol. 8 (1996) 74–85.
[3] M.H. Ginsberg, M.A. Schwartz, M.D. Schaller, Integrins: emerging paradigms of signal transduction, Ann Rev. Cell Biol. 11 (1995) 549–599.

[4] C.E. Turner, J.R. Glenney, K.B. Burridge, Paxillin; a new vinculin-binding protein present in focal adhesions, J. Cell Biol. 111 (1990) 1059–1068.

[5] C.E. Turner, J.T. Miller, Primary sequence of paxillin contains putative SH2 and SH3 domain binding motifs and multiple LIM domains: Identification of a vinculin and pp125FAK-binding region, J. Cell Sci. 107 (1994) 1583–1591.

[6] M.C. Brown, J.A. Perrotta, C.E. Turner, Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding, J. Cell Biol. 135 (1996) 1109–1123.

[7] K. Tachibana, T. Sato, N. D'Airro, C. Morimoto, Direct association of pp125FAK with paxillin, the focal adhesion-targeting mechanism of pp125FAK, J. Exp. Med. 182 (1995) 1089–1100.

[8] S.B. VandePol, M.C. Brown, C.E. Turner, Association of bovine papilloma virus Type 1 E6 oncoprotein with the focal adhesion protein paxillin through a conserved protein interaction motif, Oncogene 16 (1998) 43–52.

[9] Z. Weng, J.A. Taylor, C.E. Turner, J.S. Brugge, C. Seidel-Duggan, Detection of Src-SH3 binding proteins, including paxillin, in normal and v-src transformed BALB/c3T3 cells, J. Biol. Chem. 268 (1993) 14956–14963.

[10] C.E. Turner, Paxillin; a cytoskeletal target for tyrosine kinases, BioEssays 16 (1994) 47–52.

[11] E. Rozengurt, Convergent signalling in the action of integrins, neuropeptides, growth factors and hormones, Cancer Surveys 24 (1995) 81–96.

[12] S.L. Bellis, J.T. Miller, C.E. Turner, Characterization of tyrosine phosphorylation of paxillin in vitro by focal adhesion kinase, J. Biol. Chem. 270 (1995) 17437–17441.

[13] M.D. Schaller, J.T. Parsons, pp125FAK-dependent phosphorylation of paxillin creates a high affinity binding site for Crk, Mol. Cell Biol. 15 (1995) 2635–2645.

[14] S.L. Bellis, J.A. Perrotta, M.S. Curtis, C.E. Turner, Adhesion of fibroblasts to fibronectin stimulates both serine and tyrosine phosphorylation of paxillin, Biochem. J. 325 (1997) 375–381.

[15] M.C. Brown, J.A. Perrotta, C.E. Turner, Serine and threonine phosphorylation of the paxillin LIM domains regulates paxillin focal adhesion localization and cell adhesion to fibronectin, Mol. Biol. Cell. (1998), in press.

[16] R. Yamaguchi, Y. Mazaki, K. Hiorta, S. Hashimoto, H. Sabe, Mitosis specific serine phosphorylation and down-regulation of one of the focal adhesion proteins, paxillin, Oncogene 15 (1997) 1753–1761.

[17] M. Matsuya, H. Sasaki, H. Aoto, T. Mitaka, K. Nagura, T. Ohba, M. Ishino, S. Takahashi, R. Suzuki, T. Sasaki, Cell adhesion kinase β forms a complex with a new member, Hic-5, of proteins localized at focal adhesions, J. Biol. Chem. 273 (1998) 1003–1014.

[18] R. Salgia, J.-L. Li, S.H. Lo, B. Brunkhorst et al, Molecular cloning of human paxillin, a focal adhesion protein phosphorylated by p210BCR/ABL, J. Biol. Chem. 270 (1995) 5039–5047.

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Nonreceptor protein tyrosine kinase involvement in signal transduction and immunodeficiency disease .

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The nonreceptor **protein tyrosine kinases** (PTKs) have been grouped into 10 different enzyme families based on predicted amino acid sequences. As the number of enzymes belonging to the nonreceptor class of PTK is increasing, one challenge is to **determine** how these various classes of PTKs interact within the cell to promote **signal transduction** . Herein, the activation of four classes of nonreceptor PTKs is discussed in relation to their interactions with each other as well as with other signaling molecules during the process of lymphocyte surface antigen receptor-mediated activation. Recent findings of nonreceptor PTK loss-of-function mutations in different immunodeficiency diseases has revealed the important contribution of this group of enzymes to lymphocyte development. (65 Refs.)

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Signal transduction interception as a novel approach to disease management.

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Mechanisms of Disease: The Thyrotropin Receptor in Thyroid Diseases (Review Article)

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TEXT

The growth and function of the thyroid are controlled by thyrotropin (Ref. 1) through the activation of its receptor, which belongs to the large family of G protein-coupled receptors. Despite the extreme diversity of their ligands, all receptors from this family have a common molecular architecture: seven transmembrane segments, three extracellular loops, three intracellular loops, an extracellular amino terminal, and an intracytoplasmic carboxy terminal (Fig. 1). The glycoprotein hormone receptors constitute a subfamily that is characterized mainly by a particularly long amino-terminal extracellular domain that confers binding specificity. (Ref. 1,21)|*Figure 1.-The Thyrotropin Receptor.The location of constitutively activating mutations (Ref. 1-16) and inactivating mutations (Ref. 15,17,18) of the thyrotropin-receptor gene is shown, as is the location of somatic mutations found in thyroid carcinomas. (Ref. 10,19,20) At some locations, several different amino acid substitutions have been described. All gain-of-function mutations are in exon 10 except Ser281Asn/Thr, which is in exon 9. Gain-of-function mutations are denoted by circles in the case of hyperfunctioning thyroid adenomas, squares in the case of familial autosomal dominant hyperthyroidism, diamonds in the case of sporadic congenital hyperthyroidism, and octagons in the case of thyroid carcinomas. Loss-of-function mutations are denoted by triangles. Letters indicate the amino acid in the wild-type receptor. The asterisk and double asterisk indicate deletions resulting in a gain of function in hyperfunctioning thyroid adenomas *.**FIGURE OMITTED**

The thyrotropin receptor is encoded by 10 exons spread over 58 kilobases on chromosome 14. The large extracellular domain is encoded by the first nine exons, and the transmembrane segments and the carboxy terminal are encoded by exon 10. A number of splice variants of the receptor have been described, but their pathophysiologic importance is not known. (Ref. 1,21,22)

The thyrotropin receptor is preferentially coupled to the (alpha) subunit of the stimulatory guanine-nucleotide-binding protein (G(sub s)(alpha)) that activates adenylate cyclase and increases the accumulation of cyclic AMP (cAMP). At higher thyrotropin concentrations, the receptor also couples to the q subunit of guanine-nucleotide-binding protein alpha, resulting in the activation of phospholipase C, and there is recent evidence that the receptor may be coupled to members of other G protein families. (Ref. 23) In addition, insulin-like growth factor I, epidermal growth factor, transforming growth factor (beta), platelet-derived growth factor, fibroblast growth factor, and cytokines, mainly acting by means of the protein tyrosine kinase signal-transduction pathway, stimulate the growth and dedifferentiation of thyroid epithelial cells. (Ref. 1)

Both the growth and the function of the thyroid are stimulated by cAMP. (Ref. 1,24) This second messenger indirectly regulates the expression

of the thyroglobulin and thyroid peroxidase genes, whose promoters contain binding sites for the transcription factors TTF1, TTF2, and PAX8. (Ref. 25) As a consequence, continued stimulation of the cAMP pathway causes hyperthyroidism and thyroid hyperplasia (Fig. 2). The best examples of this are provided by Graves' disease, in which autoantibodies mimic the action of thyrotropin, (Ref. 26) and transgenic mice whose thyroids express the A2 adenosine receptor. (Ref. 27)|*Figure 2.-Consequences of the Activation of the Thyrotropin Receptor.The thyrotropin receptor is coupled mainly to the cAMP pathway by means of the (alpha) subunit of the stimulatory guanine-nucleotide-binding protein. The cAMP pathway regulates the production of thyroid hormone and the proliferation of thyroid epithelial cells and thereby mediates hyperthyroidism as well as the formation of thyroid adenomas. Both hormone production and the growth of thyroid epithelial cells are either stimulated (up arrow) or inhibited (down arrow) by local factors, such as insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), and transforming growth factor (beta) (TGF-(beta)) *.**FIGURE OMITTED**

Therefore, if a somatic mutation in a single thyroid epithelial cell caused chronic stimulation of the cAMP pathway, that cell would acquire a growth advantage and clonal expansion would result, leading to the formation of an autonomously functioning thyroid adenoma and ultimately to hyperthyroidism. Conversely, inhibition of this cascade by autoantibodies that block the thyrotropin receptor or by a defective thyrotropin receptor would result in hypothyroidism. In recent years alterations of the thyrotropin receptor and the regulatory pathways it controls have been identified in both hyperthyroidism and hypothyroidiAutonomously Functioning Thyroid Adenomas

In areas where there is sufficient iodine, hyperthyroidism due to Graves' disease is 50 times as prevalent as hyperthyroidism caused by an autonomously functioning thyroid adenoma. (Ref. 28) In contrast, in iodine-deficient areas, the causes of hyperthyroidism are different. For example, in East Jutland (Denmark) hyperthyroidism was caused by Graves' disease in 39 percent of patients, by autonomously functioning thyroid adenomas in 10 percent, and by toxic multinodular goiters in 48 percent. (Ref. 29) Therefore, iodine deficiency appears to promote the development of autonomously functioning thyroid adenomas.

These adenomas synthesize and secrete thyroid hormones autonomously, thereby suppressing thyrotropin secretion, so that the extranodular tissue becomes quiescent. On radionuclide imaging of the thyroid the adenomas are hyperfunctioning (``hot''), as compared with the paranodular thyroid tissue, which is deprived of thyrotropin stimulation. Depending on the iodine intake, growth potential, and other variables, it may take months to a decade or longer for an adenoma to grow large enough to cause hyperthyroidism. (Ref. 30)

The coexistence of autonomous and quiescent tissue in the thyroid gland suggests an inherent defect as the cause of autonomously functioning adenomas. This assumption is supported by the persistence of hyperactivity of adenoma cells in cell culture and after grafting into nude mice. (Ref. 31) Moreover, the occurrence of hyperthyroidism together with thyrotropin-independent growth of the adenoma suggests chronic activation of the cAMP cascade (Fig. 2). Somatic mutations in a gene of the cAMP regulatory cascade leading to constitutive activation (e.g., activation in the absence of stimulatory ligand) of this cascade were first detected in G(sub s)(alpha) in pituitary somatotroph adenomas. (Ref. 32) Subsequently, G(sub s)(alpha) mutations were identified in 12 to 38 percent of autonomously functioning thyroid adenomas. (Ref. 33)

G protein-coupled receptors have extensive homology in the transmembrane regions involved in receptor activation, as demonstrated by studies of site-directed mutagenesis with another G protein-coupled receptor, the (alpha)1b-adrenergic receptor. (Ref. 34) Therefore, it seemed logical to screen the same region of the thyrotropin-receptor gene for somatic mutations in autonomously functioning thyroid adenomas. The first mutations were identified in the third intracellular loop of the receptor (Ref. 2) (Fig. 1), but mutations in other regions were subsequently detected. (Ref. 2-4) Some of the affected patients were clinically euthyroid, but most had hyperthyroidism. To date, 28 substitutions in 21 amino acid residues conferring constitutive activity to the thyrotropin

receptor have been identified (including those found in patients with familial or sporadic congenital nonautoimmune hyperthyroidism) (Fig. 1). When the function of these mutants was assessed by transfection of recombinant constructs into COS cells, they all increased thyrotropin-independent cAMP production over that induced by the wild-type receptor. (Ref. 1,2-12) Constitutively activating mutations were also identified in the genes for the luteinizing hormone receptor in boys with precocious puberty, (Ref. 35) the parathyroid hormone receptor in patients with Jansen's metaphyseal chondrodysplasia, (Ref. 36) and the follicle-stimulating hormone receptor in a man with sustained spermatogenesis who had undergone hypophysectomy. (Ref. 37) Constitutively activating mutations in G protein-coupled receptors are therefore emerging as a new pathophysiologic entity in endocrinology.

The frequency of mutations in the thyrotropin-receptor gene in autonomously functioning thyroid adenomas varies with the sensitivity of the detection method used (direct sequencing being more sensitive than analysis involving single-strand conformation polymorphisms), the extent of the region screened for mutations, the quality of the tissue examined (mutations are more difficult to find in highly fragmented DNA extracted from paraffin-embedded tissue than in frozen tissue), and the type of tissue sampling (surgical specimens vs. those obtained by fine-needle aspiration biopsy). In addition, other factors such as the genetic background and the iodine intake might influence the incidence of thyrotropin-receptor gene mutations in the adenomas. In studies reporting low frequencies (0 or 8 percent) of mutations in the thyrotropin-receptor gene, only parts of exon 10 were studied, (Ref. 10,38) whereas in studies reporting high frequencies (48 to 80 percent) nearly all of exon 10 was sequenced. (Ref. 4,12,13,39) In a study in which all of exon 10 was sequenced in 44 adenomas, the incidence of mutations was 20 percent. (Ref. 40) However, in the same study G(sub s)(alpha) mutations were found in 24 percent of the adenomas. In two other studies in which the incidence of mutations in exon 10 of the thyrotropin-receptor gene was high (70 and 48 percent), the incidence of G(sub s)(alpha) mutations was low (0 and 4 percent, respectively). (Ref. 12,13) Mutations leading to constitutive activation of the cAMP cascade appear to be the cause of a substantial proportion (48 to 70 percent) of autonomously functioning thyroid adenomas.

Mutations in the thyrotropin-receptor gene were detected in 5 of 12 hyperfunctioning nodules in six patients with toxic multinodular goiters, each of whom had 2 hyperfunctioning nodules. (Ref. 8) Furthermore, cAMP-independent pathways have been implicated in goitrogenesis, (Ref. 24) suggesting the action of different, partly overlapping pathophysiologic mechanisms in the heterogeneous disorder of toxic multinodular goiter.

Differentiated Thyroid Cancer

The demonstration of the oncogenic potential of the cAMP pathway in benign hyperfunctioning thyroid adenomas prompted investigation of the role of this pathway in the pathogenesis of differentiated thyroid carcinomas. Mutations in G(sub s)(alpha) were found in 7 of 61 carcinomas in two studies. (Ref. 41,42) In two other studies, constitutively activating mutations in the thyrotropin-receptor gene were detected in 5 of 44 carcinomas (Ref. 19,20); no G(sub s)(alpha) mutations were found in any of the tumors. All the mutations in the thyrotropin-receptor gene in these tumors had previously been identified in autonomously functioning adenomas (Fig. 1). Moreover, in one patient a thyroid carcinoma with a somatic mutation resulted in the production of sufficient thyroid hormone to cause hyperthyroidism. (Ref. 20)

Constitutive activation of the cAMP pathway in the thyroid leads to progressive growth of thyrocytes, while usually maintaining cell differentiation. (Ref. 24) However, the thyroid carcinomas with G(sub s)(alpha) mutations did not take up iodine, (Ref. 19) suggesting a second, dedifferentiating genetic alteration. In agreement with a multistep model of thyroid carcinogenesis, the presence of a G(sub s)(alpha) or a thyrotropin-receptor gene mutation together with an activated ras gene has been demonstrated in differentiated carcinoma. (Ref. 19) It is therefore likely that both the ras and G(sub s)(alpha) or thyrotropin-receptor gene mutations act synergistically to cause the tumor phenotype in some differentiated thyroid carcinomas. However, thyrotropin-receptor gene or G(sub s)(alpha) mutations in differentiated thyroid carcinomas are rare,

and chronic activation of the cAMP pathway probably does not increase the likelihood of further genetic damage, as suggested by the rarity of malignant transformation in autonomously functioning thyroid adenomas, Graves' disease, and the benign toxic thyroid hyperplasia in transgenic mice with chronic stimulation of the cAMP pathway by ectopic expression of the adenosine A2 receptor (Ref. 27) or G(sub s)(alpha) mutations (Ref. 43) in the thyroid gland.

Germ-line Mutations in the Thyrotropin Receptor in Autosomal Dominant Nonautoimmune Hyperthyroidism

Familial clustering of hyperthyroidism due to Graves' disease is a well-known phenomenon. Autosomal dominant inheritance of nonautoimmune hyperthyroidism was described 15 years ago. (Ref. 44) Once somatic mutations in the thyrotropin-receptor gene and G(sub s)(alpha) had been demonstrated in autonomously functioning thyroid adenomas, families with nonautoimmune hypothyroidism were reevaluated for germ-line mutations in the thyrotropin-receptor gene.

Sequencing of the thyrotropin-receptor gene in two families with hereditary nonautoimmune hyperthyroidism led to the identification of constitutively activating heterozygous germ-line mutations. (Ref. 45) The functional in vitro characteristics of these two mutations were similar to those already described for autonomously functioning thyroid adenomas, (Ref. 1) and thus explain the development of thyroid hyperplasia and hyperthyroidism in affected patients.

Six other families with different mutations in the thyrotropin-receptor germ line have subsequently been identified. (Ref. 7,9,11,46) These patients do not have the clinical manifestations of hyperthyroidism due to Graves' disease, such as thyroid-associated ophthalmopathy, pretibial myxedema, or lymphocytic infiltration of the thyroid, nor do they have any thyroid antibodies. The thyroid gland is enlarged in most patients. Hyperthyroidism can occur at any time from the neonatal period to adulthood. This variability in the age at onset is probably the result of other genetic components and environmental factors such as iodine intake and dietary goitrogens. Patients require ablative treatment (surgery or radioiodine), because recurrent hyperthyroidism after subtotal thyroidectomy, mandating a second thyroidectomy or radioiodine treatment, has been reported in many families.

Germ-line mutations can be inherited, or they can arise spontaneously. In four infants with sporadic congenital hyperthyroidism, germ-line mutations in the thyrotropin-receptor gene were identified. (Ref. 9,14,17,47) All four infants had severe, persistent hyperthyroidism without thyroid antibodies. In all cases, both parents were euthyroid and none had germ-line mutations in the thyrotropin-receptor gene or Graves' disease. These patients can therefore be classified as having sporadic congenital nonautoimmune autosomal dominant hyperthyroidism. Two of the four patients were treated by thyroidectomy because of persistent hyperthyroidism during treatment with an antithyroid drug and rapidly enlarging goiters. (Ref. 17,47)

As shown in Figure 1, a number of mutations in the thyrotropin-receptor gene have been found both as somatic mutations in patients with autonomously functioning thyroid adenomas and as germ-line mutations in patients with autosomal dominant nonautoimmune hyperthyroidism. This is convincing evidence of a common pathophysiologic mechanism. Moreover, sporadic nonautoimmune hyperthyroidism can eventually become an inherited disease. Very early onset of hyperthyroidism has been reported in two women whose children had congenital nonautoimmune hyperthyroidism. (Ref. 12,46)

The question of whether the various mutations in the thyrotropin-receptor gene cause different activities of the cAMP pathway was addressed by preliminary parallel in vitro studies of 11 mutations. (Ref. 1) The basal activities of the mutations varied widely. Most of the mutations activated only the cAMP cascade, but five (I486M, A623I, I568T, T632I, and I486F) also activated the phospholipase C-dependent cascade. However, the in vitro activities of the somatic and germ-line mutations were similar, and there is no apparent phenotypic difference between patients with different mutations in hot nodules or families with germ-line mutations in well-conserved as opposed to nonconserved amino acid residues.

Nonetheless, some preliminary diagnostic and therapeutic conclusions

can be drawn on the basis of the clinical characteristics of the patients with germ-line mutations in the thyrotropin-receptor gene. In families in which multiple members have nonautoimmune hyperthyroidism and in persons with sporadic congenital hyperthyroidism and no evidence of an autoimmune cause, a search for mutations in the thyrotropin-receptor gene is indicated. Only the identification of a mutation will lead to a definitive diagnosis. Patients with germ-line mutations should be treated early by removing as much thyroid tissue as possible to control the hyperthyroidism permanently and avoid relapses.

Thyrotropin Resistance

Several reports of patients with nonautoimmune congenital hypothyroidism have suggested that there is a syndrome of resistance of the thyroid to thyrotropin. (Ref. 48-50) All the affected patients have had normal-sized thyroid glands but high serum concentrations of biologically active thyrotropin. The absence of thyroglobulin in the thyroid gland in one patient and a decreased uptake of radioiodine by the thyroid in another patient are consistent with an impaired cAMP-signaling system as the cause of reduced synthesis of thyroid-specific proteins and impaired proliferation of thyroid epithelial cells (Fig. 2). Additional evidence of a putative abnormality in the thyrotropin-receptor-adenylate cyclase system was provided by the demonstration of thyrotropin unresponsiveness both in vivo and in vitro. (Ref. 49,50)

Thyrotropin resistance can be defined as reduced responsiveness or nonresponsiveness of the thyroid gland to biologically active thyrotropin. It can be caused by defects in the receptor itself or in elements involved in the transduction of signals from the receptor into the cell. Contrary to the dominant gain-of-function mutations in the thyrotropin-receptor gene in autonomously functioning thyroid adenomas, which are heterozygous (only one mutated allele), loss-of-function mutations probably require mutations in both alleles in order to be expressed, unless the product of the mutated allele interferes with the expression of the wild-type allele. In keeping with this hypothesis, the first loss-of-function point mutation in the thyrotropin-receptor gene, which was identified in hypothyroid (hyt/hyt) mice, affected both alleles. (Ref. 51)

The molecular basis of thyrotropin resistance in humans was recently identified in three siblings who were euthyroid and had normal serum thyroid hormone concentrations but high serum thyrotropin concentrations. All three patients were compound heterozygotes, each having a point mutation in each thyrotropin-receptor allele; the heterozygous parents were asymptomatic. Whereas the paternal allele (Ile167Ala) had completely lost the capacity to be activated by thyrotropin, the maternal allele (Pro162Ala) had only reduced sensitivity to the hormone. (Ref. 52) Subsequently, six more families with thyrotropin resistance have been identified. (Ref. 15,18,53,54) The majority of the probands were identified on the basis of elevated blood thyrotropin concentrations at the time of neonatal screening. However, in contrast to patients with congenital hypothyroidism, nearly all the patients had normal serum thyroid hormone concentrations. (Ref. 15) Their thyroid glands were normal-sized, a hypoplastic thyroid gland being reported in only one patient. (Ref. 18) However, in three patients with congenital hypothyroidism and no thyroid enlargement who were unresponsive to thyrotropin, no mutation in the thyrotropin-receptor gene was found. (Ref. 55) Therefore, defects in other genes or flanking regions of the thyrotropin receptor might also cause thyrotropin resistance.

The Thyrotropin Receptor and Autoimmunity

In addition to its role in nonautoimmune hyperthyroidism and hypothyroidism, the thyrotropin receptor is also an autoantigen. However, in this case the receptor seems to be a bystander, because mutations in the receptor originally thought to be implicated in patients with Graves' disease have been found in their normal relatives and in unrelated **normal** subjects. (Ref. 1,22)

The thyrotropin receptor is the target of both thyroid-stimulating antibodies (as in Graves' hyperthyroidism) and thyroid-blocking antibodies (as in chronic autoimmune thyroiditis). The majority of thyroid-stimulating antibodies and thyroid-blocking antibodies are also immunoglobulins that inhibit the binding of thyrotropin. (Ref. 56) Both types of antibodies may coexist in the same patient. (Ref. 26)

Unlike other antibodies (e.g., antithyroperoxidase and antithyroglobulin antibodies), (Ref. 57) thyrotropin-receptor antibodies are directly involved in the pathogenesis of Graves' disease, as demonstrated by the occurrence of transient hyperthyroidism in neonates whose mothers have thyroid-stimulating antibodies. (Ref. 26) However, serum obtained from a minority of untreated patients with hyperthyroidism due to Graves' disease contains no **detectable** thyrotropin-receptor antibodies. This may be due to the insensitivity of the current **assays** or because these patients do not have Graves' disease.

In an attempt to understand why thyrotropin-receptor antibodies may be thyrotropin agonists and with the aim of developing improved **assays** for these antibodies, considerable effort has been spent to define the regions of the thyrotropin receptor involved in the binding and bioactivity of thyrotropin, thyrotropin-receptor-stimulating antibodies, and thyrotropin-receptor-binding antibodies. The main findings are that both thyrotropin and thyrotropin-receptor antibodies bind to numerous discontinuous residues throughout the extracellular region of the receptor (i.e., their binding sites and their bioactivity depend on the recognition of the three-dimensional structure of the thyrotropin receptor). (Ref. 58,59) The finding that there is binding to different regions of the thyrotropin receptor provides some explanation for the existence of antibodies having agonist or antagonist activities, but further progress in this area awaits the elucidation of the three-dimensional structure of the receptor and of the mechanism of receptor activation.

Conclusions

The thyrotropin receptor is involved in a wide range of sporadic and hereditary or genetically **determined** changes in thyroid function, most likely because small changes in amino acid composition readily alter its configuration, resulting in increased intrinsic activity or decreased responsiveness to thyrotropin. In Graves' disease the thyrotropin receptor is the target of thyroid-stimulating and thyroid-blocking antibodies.

This article is dedicated to the memory of Olaf Heine, M.D

CITED REFERENCES

1. Van Sande J, Parma J, Tonacchera M, Swillens S, Dumont J, Vassart G. Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab 1995;80:2577-85.
2. Parma J, Duprez L, Van Sande J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature 1993;365:649-51.
3. Paschke R, Tonacchera M, Van Sande J, Parma J, Vassart G. Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. J Clin Endocrinol Metab 1994;79:1785-9.
4. Porcellini A, Ciullo I, Laviola L, Amabile G, Fenzi G, Avvedimento VE. Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas: rapid identification by fine needle aspiration biopsy. J Clin Endocrinol Metab 1994;79:657-61.
5. Kosugi S, Shenker A, Mori T. Constitutive activation of cyclic AMP but not phosphatidylinositol signaling caused by four mutations in the 6th transmembrane helix of the human thyrotropin receptor. FEBS Lett 1994;356:291-4.
6. Tonacchera M, Van Sande J, Cetani F, et al. Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. J Clin Endocrinol Metab 1996;81:547-54.
7. Fuhrer D, Willgerodt H, Scherbaum WA, Paschke R. Identification of a new germline mutation in the thyrotropin receptor gene as a cause of familial nonautoimmune hyperthyroidism. J Clin Endocrinol Metab (in press).
8. Holzapfel HP, Fuhrer D, Wonerow P, Weinland G, Scherbaum WA, Paschke R. Identification of constitutively activating somatic thyrotropin receptor mutations in a subset of toxic multinodular goitres. J Clin Endocrinol Metab (in press).
9. De Roux N, Polak M, Couet J, et al. A neomutation of the

thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism. J Clin Endocrinol Metab 1996;81:2023-6.
10. Russo D, Arturi F, Wicker R, et al. Genetic alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab 1995;80:1347-51.
11. Kohler B, Biebermann H, Krohn HP, Dralle D, Finke R, Gruters A. A novel germline mutation in the thyrotropin receptor gene causing nonautoimmune congenital hyperthyroidism. In: Volume I (June 12-13) of program & abstracts of the 10th International Congress of Endocrinology (ICE '96), San Francisco, June 12-15, 1996. Bethesda, Md.: Endocrine Society Press, 1996:641. abstract.
12. Fuhrer D, Holzapfel HP, Wonerow P, Scherbaum WA, Paschke R. Screening for mutations in the thyrotropin receptor and gsp genes in toxic thyroid nodules. J Clin Endocrinol (in press).
13. Parma J, Duprez L, Van Sande J, et al. Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs (alpha) genes as a cause of toxic thyroid adenomas. J Clin Endocrinol Metab 1997;82:2695-701.
14. Esapa C, Betts P, Kendall-Taylor P, Harris PE. A novel TSH receptor mutation in an infant with thyrotoxicosis. J Endocrinol Invest 1996;19:Suppl 6:71. abstract.
15. Clifton-Bligh RJ, Gregory JW, Ludgate R, et al. Two novel mutations in the thyrotropin (TSH) receptor gene in a child with resistance to TSH. J Clin Endocrinol Metab 1996;82:1094-100.
16. Russo D, Belfiore A, Tumino S, et al. A thyroid follicular cancer presenting as a hot nodule and hyperfunctioning because of an activating mutation of the TSH receptor. J Endocrinol Invest 1996;19:Suppl 6:1. abstract.
17. Kopp P, van Sande J, Parma J, et al. Congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. N Engl J Med 1995;332:150-4.
18. Biebermann H, Krude H, Thiede C, Kotulla D, Gruters A. Sporadic congenital hypothyroidism due to compound heterozygosity for two mutations of the coding sequence of the thyrotropin receptor gene. In: Volume I (June 12-13) of program & abstracts of the 10th International Congress of Endocrinology (ICE '96), San Francisco, June 12-15, 1996. Bethesda, Md.: Endocrine Society Press, 1996:643. abstract.
19. Russo D, Arturi F, Schlumberger M, et al. Activating mutations of the TSH receptor in differentiated thyroid carcinomas. Oncogene 1995;11:1907-11.
20. Spambalg D, Sharifi N, Elisi R, Gross JL, Medeiros-Neto G, Fagin JA. Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. J Clin Endocrinol Metab 1996;81:3898-901.
21. Paschke R, Van Sande J, Parma J, Vassart G. The TSH receptor and thyroid disease. Baillieres Clin Endocrinol Metab 1996;10:9-27.
22. Paschke R, Vassart G, Ludgate M. Current evidence for and against the TSH receptor being the common antigen in Graves' disease and thyroid associated ophthalmopathy. Clin Endocrinol (Oxf) 1995;42:565-9.
23. Laugwitz K-L, Allgeier A, Offermanns S, et al. The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. Proc Natl Acad Sci U S A 1996;93:116-20.
24. Dumont JE, Maenhaut C, Pirson I, Baptist M, Roger PP. Growth factors **controlling** the thyroid gland. Baillieres Clin Endocrinol Metab 1991;5:727-54.
25. Damante G, DiLauro R. Thyroid-specific gene expression. Biochim Biophys Acta 1994;1218:255-66.
26. McKenzie JM, Zakarija M. The clinical use of thyrotropin receptor antibody **measurements** . J Clin Endocrinol Metab 1989;69:1093-6.
27. Ledent C, Dumont JE, Vassart G, Parmentier M. Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J 1992;11:537-42.
28. Hamburger JI. Evolution of toxicity in solitary nontoxic autonomously functioning thyroid nodules. J Clin Endocrinol Metab 1980;50:1089-93.
29. Laurberg P, Pedersen KM, Vestergaard H, Sigurdsson G. High incidence of multinodular toxic goitre in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a

high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland. J Intern Med 1991;229:415-20.
30. Sandrock D, Olbricht T, Emrich D, Benker G, Reinwein D. Long-term follow- up in patients with autonomous thyroid adenoma. Acta Endocrinol (Copenh) 1993;128:51-5.
31. Usadel KH, Paschke R, Teuber J, Schwedes U. Hetero-transplantation of autoimmune human thyroid to nude mice as a tool for in vivo autoimmune research. In: Pinchera A, Ingbar SH, McKenzie JM, Fenzi GF, eds. Thyroid autoimmunity. New York: Plenum Press, 1987:207-10.
32. Lyons J, Landis CA, Harsh G, et al. Two G protein oncogenes in human endocrine tumors. Science 1990;249:655-9.
33. Spiegel AM, Weinstein LS, Shenker A. Abnormalities in G protein-coupled **signal transduction** pathways in human disease. J Clin Invest 1993;92:1119-25.
34. Kjelsberg MA, Cotecchia S, Ostrowski J, Caron MG, Lefkowitz RJ. Constitutive activation of the (alpha) 1B-adrenergic receptor by all amino acid substitutions at a single site: evidence for a region which constrains receptor activation. J Biol Chem 1992;267:1430-3.
35. Shenker A, Laue L, Kosugi S, Merendino JJ Jr, Minegishi T, Cutler GB Jr. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. Nature 1993;365:652-4.
36. Schipani E, Langman CB, Parfitt AM, et al. Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia. N Engl J Med 1996;335:708-14.
37. Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. J Clin Endocrinol Metab 1996;81:1367-70.
38. Takeshita A, Nagayama Y, Yokoyama N, et al. Rarity of oncogenic mutations in the thyrotropin receptor of autonomously functioning thyroid nodules in Japan. J Clin Endocrinol Metab 1995;80:2607-11.
39. Parma J, Van Sande J, Swillens S, Tonacchera M, Dumont JE, Vassart G. Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3',5'-monophosphate and inositol phosphate-Ca2+ cascades. Mol Endocrinol 1995;9:725-33.
40. Russo D, Arturi F, Suarez HG, et al. Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab 1996;81:1548-51.
41. Suarez HG, du Villard JA, Caillou B, Schlumberger M, Parmentier C, Monier R. Gsp mutations in human thyroid tumours. Oncogene 1991;6:677-9.
42. Yoshimoto K, Iwahana H, Fukuda A, Sano T, Itakura M. Rare mutations of the Gs alpha subunit gene in human endocrine tumors: mutation **detection** by polymerase chain reaction-primer-introduced restriction analysis. Cancer 1993;72:1386-93.
43. Michiels FM, Caillou B, Talbot M, et al. Oncogenic potential of guanine nucleotide stimulatory factor alpha subunit in thyroid glands of transgenic mice. Proc Natl Acad Sci U S A 1994;91:10488-92.
44. Thomas JS, Leclere J, Hartemann P, et al. Familial hyperthyroidism without evidence of autoimmunity. Acta Endocrinol (Copenh) 1982;100:512-8.
45. Duprez L, Parma J, Van Sande J, et al. Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. Nat Genet 1994;7:396-401.
46. Schwab KO, Sohlemann P, Gerlich M, et al. Mutations of the TSH receptor as a cause of congenital hyperthyroidism. Exp Clin Endocrinol Diabetes 1996;104:Suppl 4:124-8.
47. Holzapfel H-P, Wonerow P, Petrykowski W, Henschen M, Scherbaum WA, Paschke R. Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. J Clin Endocrinol Metab (in press).
48. Takamatsu J, Nishikawa M, Horimoto M, Ohsawa N. Familial unresponsiveness to thyrotropin by autosomal recessive inheritance. J

Clin Endocrinol Metab 1993;77:1569-73.
49. Codaccioni JL, Carayon P, Michel-Bechet M, Foucault F, Lefort G, Pierron H. Congenital hypothyroidism associated with thyrotropin unresponsiveness and thyroid cell membrane alterations. J Clin Endocrinol Metab 1980;50:932-7.
50. Medeiros-Neto GA, Knobel M, Bronstein MD, Simonetti J, Filho FF, Mattar E. Impaired cyclic-AMP response to thyrotropin in congenital hypothyroidism with thyroglobulin deficiency. Acta Endocrinol (Copenh) 1979;92:62-72.
51. Stein SA, Oates EL, Hall CR, et al. Identification of a point mutation in the thyrotropin receptor of the hyt/hyt hypothyroid mouse. Mol Endocrinol 1994;8:129-38.
52. Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, Refetoff S. Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. N Engl J Med 1995;332:155-60.
53. Asteria C, Persani L, Romoli R, Beck-Peccoz P. Resistance to thyrotropin action resulting from inactivating mutation of thyrotropin receptor (TSH-R) gene. J Endocrinol Invest 1996;19:Suppl 6:26. abstract.
54. DeRoux N, Misrahi M, Brauner R, et al. Four families with loss of function mutations of the thyrotropin receptor. J Clin Endocrinol Metab 1996;81:4229-35.
55. Takeshita A, Nagayama Y, Yamashita S, et al. Sequence analysis of the thyrotropin (TSH) receptor gene in congenital primary hypothyroidism associated with TSH unresponsiveness. Thyroid 1994;4:255-9.
56. Rees Smith B, McLachlan SM, Furmaniak J. Autoantibodies to the thyrotropin receptor. Endocr Rev 1988;9:106-21.
57. Ludgate M, Vassart G. The molecular genetics of three thyroid autoantigens: thyroglobulin, thyroid peroxidase and the thyrotropin receptor. Autoimmunity 1990;7:201-11.
58. Kohn LD, Shimura H, Shimura Y, et al. The thyrotropin receptor. Vitam Horm 1995;50:287-384.
59. McLachlan S, Rapoport B. Recombinant thyroid autoantigens: the keys to the pathogenesis of autoimmune thyroid disease. J Intern Med 1993;234:347-59.

13/9/14 (Item 14 from file: 73)

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Hyperactivation of signal transduction systems in Alzheimer's disease

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A compromise or deregulation in signal transduction cascades could adversely affect cellular functions and possibly contribute to cell death. In recent years, it has become increasingly apparent that pronounced activation of neuronal signal transduction systems is a characteristic of AD brain. There is evidence that signal transduction systems play a role in the formation or development of these pathological features of AD. Aberrant activity and localization of components of signaling mechanisms (growth factors, their receptors, protein kinases, phosphoprotein phosphatases, and phosphoproteins) are closely associated with the intracellular accumulation of PHF, the extracellular deposition of amyloid, and the formation of neuritic plaques in AD brain. In particular, immunohistochemical studies reveal increased levels of neuronal staining for APP, possibly an important growth factor in AD, both in frontal cortex and hippocampus. Anti-APP immunostaining is also associated with the neuritic component of plaques. Additionally, PKC(betaII) immunostaining is increased in the neuronal cell

body and neuropil of AD samples, particularly in association with plaques, suggesting a postsynaptic involvement of this enzyme. On the other hand, PKC(betaI) immunostaining is associated with axonal staining particularly in the sprouting neurites of plaques. Sprouting neuritic components of plaques are immunopositive with other growth-associated proteins, such as GAP43, MARCKS, and spectrin. Immunoreactivity of other members of signal transduction systems such as Fos and stathmin are all increased in AD hippocampal neurons. On the other hand, several protein kinases and phosphoproteins were immunolocalized to tangles. Thus, the hyperactivation and dysfunction of signal transduction systems could be involved in the pathogenesis of AD.

DRUG DESCRIPTORS:
growth factor receptor; amyloid protein--endogenous compound--ec; casein kinase ii--endogenous compound--ec; growth associated protein--endogenous compound--ec; growth factor--endogenous compound--ec; neuromodulin --endogenous compound--ec; phosphoprotein--endogenous compound--ec; phosphoprotein phosphatase--endogenous compound--ec; phosphotyrosine --endogenous compound--ec; protamine kinase--endogenous compound--ec; protein kinase c--endogenous compound--ec; protein tyrosine kinase --endogenous compound--ec; spectrin--endogenous compound--ec; stathmin --endogenous compound--ec; transcription factor--endogenous compound--ec
MEDICAL DESCRIPTORS:
*alzheimer disease--etiology--et; *signal transduction
apoptosis; cell death; cell function; cellular distribution; conference paper; controlled study; enzyme activity; frontal cortex; hippocampus; human; human tissue; immunohistochemistry; nerve conduction; nerve fiber; neurite; neuropil; perikaryon; priority journal; etiology
CAS REGISTRY NO.: 9025-75-6 (phosphoprotein phosphatase); 21820-51-9 (phosphotyrosine); 9068-21-7 (protamine kinase); 141436-78-4 (protein kinase c); 80449-02-1 (**protein** tyrosine kinase); 12634-43-4 (spectrin); 126880-56-6 (stathmin)
SECTION HEADINGS:
  008 Neurology and Nerosurgery
  029 Clinical and Experimental Biochemistry

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02882104 Genuine Article#: MN408 Number of References: 37
Title: IL-2-INDUCED EXPRESSION OF TTK, A SERINE, THREONINE, TYROSINE KINASE, CORRELATES WITH CELL-CYCLE PROGRESSION
Author(s): SCHMANDT R; HILL M; AMENDOLA A; MILLS GB; HOGG D
Corporate Source: TORONTO GEN HOSP,DIV ONCOL RES,CCRW 3-827,200 ELIZABETH ST/TORONTO M5G 2C4/ON/CANADA/; TORONTO GEN HOSP,DIV ONCOL RES,CCRW 3-827,200 ELIZABETH ST/TORONTO M5G 2C4/ON/CANADA/
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Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: IMMUNOLOGY
Abstract: We have recently isolated the cDNA for a unique human 97-kDa kinase, TTK, by expression screening of a cDNA expression library using anti-phosphotyrosine antibodies. When expressed in Escherichia coli, TTK can phosphorylate serine, threonine, and tyrosine residues. Thus TTK appears to belong to a newly described family of kinases able to phosphorylate all three hydroxy amino acids. This family of multispecific kinases includes several other kinases involved in cell cycle progression. In support of a possible role in regulating cell cycle progression, TTK message is readily detected in rapidly proliferating tissues in vivo including testes, thymus, bone marrow, and many malignant tumors, but not in benign tissues with a low proliferative rate in vivo. To determine the effect of cell activation and cell cycle progression on TTK expression, we measured TTK mRNA and

protein levels as well as kinase activity in freshly isolated T cells or IL-2-expanded T cell blasts activated to proliferate by the addition of a variety of mitogens. TTK mRNA levels, protein levels, and kinase activity were greatly enhanced when either freshly isolated PBL or T cell blasts were activated by cross-linking the TCR complex by mitogenic lectins or by bypassing the TCR with phorbol esters and cation ionophores. Incubation with IL-2 increased TTK expression in PBL blasts, which proliferate in response to IL-2, but not in fresh PBL, which do not proliferate in response to IL-2. TTK expression was blocked by either cyclosporin A or FK520, which inhibit IL-2 production and could be recovered by the addition of exogenous IL-2. Furthermore, TTK expression was prevented by incubation of the cells with rapamycin, which blocks IL-2 signaling. Thus, TTK expression in T cells appears to be a consequence of IL2-induced cell proliferation. Agonist-induced TTK expression was a delayed event occurring 1 2 to 24 h after activation of PBL blasts and 48 to 72 h after activation of fresh PBL. TTK protein and mRNA expression increased in both fresh PBL and T cell blasts concurrently with passage of cells through S phase as indicated by [H-3]TdR incorporation and cell cycle analysis of propidium iodide-stained cells. TTK mRNA and protein levels reached a maximum as cells entered the G2 phase of the cell cycle. These results were confirmed by cell cycle blockade studies with aphidicolin and nocodazole wherein TTK protein levels are not detected in cells in G1 and are readily detectable in cells in the S and G2 phases of the cell cycle. Furthermore, changes in TTK mRNA levels in activated cells paralleled those of cyclin A, which is expressed in late S phase and in G2 and were markedly different from those of cyclin D2, which is expressed in G1. Taken together, the data suggest that TTK may play a role in IL-2-induced passage of T cells through the S and G2M phases of the cell cycle.

Identifiers--KeyWords Plus: PROTEIN-KINASE; CYCLOSPORINE-A; PHOSPHORYLATION; ACTIVATION; RECEPTOR; P34CDC2; IDENTIFICATION; PROLIFERATION; INHIBITOR; RAPAMYCIN

Research Fronts: 91-0893 002 (TYROSINE PHOSPHORYLATION; INVITRO ACTIVATION OF A MYELIN BASIC-PROTEIN MICROTUBULE-ASSOCIATED PROTEIN-2 KINASE; THREONINE RESIDUES)

91-0133 001 (RETINOBLASTOMA PROTEIN; TUMOR SUPPRESSOR GENES; P53 MUTATIONS; HUMAN PAPILLOMAVIRUS TYPE-16 E7)

91-0237 001 (GTPASE-ACTIVATING PROTEIN; KIT RECEPTOR TYROSINE **KINASE** ; SIGNAL TRANSDUCTION; TYPE-1 NEUROFIBROMATOSIS GENE; RAS SUPERFAMILY)

91-6704 001 (T-CELL ACTIVATION VIA THE T-CELL RECEPTOR; DIFFERENTIAL REGULATION; CD2 SURFACE EXPRESSION)

Cited References:

ANDERSON NG, 1990, V343, P651, NATURE
BENDAVID Y, 1991, V10, P317, EMBO J
BIERER BE, 1991, V23, P2850, TRANSPLANT P
BISCHOFF JR, 1990, V87, P4766, P NATL ACAD SCI USA
BRUGG B, 1991, V114, P735, J CELL BIOL
BUCHKOVICH K, 1989, V58, P1097, CELL
CANTRELL DA, 1984, V224, P1312, SCIENCE
DOUVILLE EMJ, 1992, V12, P2681, MOL CELL BIOL
FEATHERSTONE C, 1991, V349, P808, NATURE
FELIX MA, 1990, V346, P379, NATURE
HANKS SK, 1991, V200, P38, METHOD ENZYMOL
HOWELL BW, 1991, V11, P568, MOL CELL BIOL
HUBERMAN JA, 1981, V23, P264, CELL
HUNTER T, 1992, V70, P372, CELL
HUNTER T, 1991, V200, P1, METHOD ENZYMOL
ICELY PL, 1991, V266, P6073, J BIOL CHEM
KLAUSNER RD, 1991, V64, P875, CELL
KREK W, 1991, V10, P305, EMBO J
LALANDE M, 1990, V186, P332, EXP CELL RES
LUCAS JJ, 1992, V148, P1804, J IMMUNOL
MILLS GB, 1991, V19, P99, BIOCHEM SOC T
MILLS GB, 1992, V267, P6000, J BIOL CHEM
MILLS GB, 1986, V136, P1150, J IMMUNOL
MORENO S, 1989, V58, P361, CELL

MORRISON DK, 1989, V58, P649, CELL
PARKER LL, 1992, V89, P2917, P NATL ACAD SCI USA
PELECH SL, 1990, V68, P1297, BIOCHEM CELL BIOL
PINES J, 1991, V56, P449, COLD SPRING HARB SYM
RAY LB, 1988, V85, P3753, P NATL ACAD SCI USA
ROY LM, 1990, V61, P825, CELL
SCHMANDT R, 1993, V5, P551, BAILLIERE CLIN HAEM
SCHREIBER SL, 1992, V13, P136, IMMUNOL TODAY
SHERR CJ, 1993, V73, P1059, CELL
SIGAL NH, 1992, V10, P519, ANNU REV IMMUNOL
STERN DF, 1991, V11, P987, MOL CELL BIOL
WEISS A, 1986, V4, P593, ANNU REV IMMUNOL
ZIEVE GW, 1980, V126, P397, EXP CELL RES

13/9/27 (Item 27 from file: 5)

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Regulation of intracellular signal transduction and gene expression by stress

BOOK TITLE: Neurobiological and clinical consequences of stress: From normal adaptation to post-traumatic stress disorder

AUTHOR: Duman Ronald S
BOOK AUTHOR/EDITOR: Friedman M J (Editor); Charney D S (Editor); Deutch A Y (Editor)
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DESCRIPTORS:

MAJOR CONCEPTS: Behavior; Biochemistry and Molecular Biophysics; Cardiovascular System--Transport and Circulation; Cell Biology; Endocrine System--Chemical Coordination and Homeostasis; Enzymology--Biochemistry and Molecular Biophysics; Genetics; Membranes--Cell Biology; Metabolism; Molecular Genetics--Biochemistry and Molecular Biophysics; Nervous System--Neural Coordination; Neurology--Human Medicine, Medical Sciences; Psychiatry--Human Medicine, Medical Sciences

BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia

ORGANISMS: human (Hominidae); rat (Muridae)

COMMON TAXONOMIC TERMS: Humans; Primates; Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates

CHEMICALS & BIOCHEMICALS: CYCLIC AMP; CYCLIC GMP; CALCIUM; NITRIC OXIDE ; ARACHIDONIC ACID; PROTEIN KINASE; **PROTEIN** TYROSINE KINASE; PROTEIN PHOSPHATASE

MISCELLANEOUS TERMS: ANIMAL MODEL; ARACHIDONIC ACID METABOLITE; BEHAVIOR; BOOK CHAPTER; BRAIN; CALCIUM; CEREBRAL CORTEX; CYCLIC AMP; CYCLIC GMP; DORSAL RAPHE; GLUCOCORTICOID; HYPOTHALAMIC-PITUITARY-ADRENAL AXIS; IMMEDIATE-EARLY GENE TRANSCRIPTION FACTOR; LOCUS COERULEUS; NERVE GROWTH FACTOR; NITRIC OXIDE; PHOSPHATIDYLINOSITOL; PHOSPHOPROTEIN; POST-TRAUMATIC STRESS DISORDER; PROTEIN PHOSPHATASE; PROTEIN PHOSPHORYLATION; PROTEIN TYROSINE KINASE;

RECEPTOR-COUPLED SECOND MESSENGER SYSTEM; SECOND MESSENGER-DEPENDENT PROTEIN KINASE; SECOND MESSENGER-INDEPENDENT PROTEIN KINASE; VENTRAL TEGMENTUM

CONCEPT CODES:
02506 Cytology - Animal
03506 Genetics - Animal
03508 Genetics - Human
07003 Behavioral biology - Animal behavior
07004 Behavioral biology - Human behavior
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10064 Biochemistry studies - Proteins, peptides and amino acids
10066 Biochemistry studies - Lipids
10067 Biochemistry studies - Sterols and steroids
10069 Biochemistry studies - Minerals
10300 Replication, transcription, translation
10506 Biophysics - Molecular properties and macromolecules
10508 Biophysics - Membrane phenomena
10612 External effects - Physical and mechanical effect
10808 Enzymes - Physiological studies
12008 Physiology - Stress
13006 Metabolism - Lipids
13008 Metabolism - Sterols and steroids
13010 Metabolism - Minerals
13012 Metabolism - Proteins, peptides and amino acids
13014 Metabolism - Nucleic acids, purines and pyrimidines
14504 Cardiovascular system - Physiology and biochemistry
17004 Endocrine - Adrenals
17014 Endocrine - Pituitary
17020 Endocrine - Neuroendocrinology
20502 Nervous system - Anatomy
20504 Nervous system - Physiology and biochemistry
20506 Nervous system - Pathology
21002 Psychiatry - Psychopathology, psychodynamics and therapy
28002 Laboratory animals - General

BIOSYSTEMATIC CODES:
86215 Hominidae
86375 Muridae

13/9/29 (Item 29 from file: 35)

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ALTERATIONS IN TYROSINE KINASE DEPENDENT SIGNAL TRANSDUCTION CORRELATE WITH FUNCTIONAL ANERGY IN T LYMPHOCYTES

Author: CHO, EUN AH
Degree: PH.D.
Year: 1994
Corporate Source/Institution: UNIVERSITY OF PENNSYLVANIA (0175)
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Descriptor Codes: 0982; 0379; 0487

Normal CD4$\sp{+}$ Th1 clones can become functionally inactivated by ligation of T cell antigen receptor (TCR/CD3 complexes in the absence of functional antigen presenting cells (APCs). These cells show proliferative nonresponsiveness, or anergy, in response to antigen restimulation with functional APCs and this nonresponsiveness is due to the lack of IL-2 production. Recently, signal transduction through tyrosine phosphorylation has been suggested to be important in T cell activation. We have shown that anergic Th1 cells express altered levels of protein tyrosine kinases: a decrease in p56$\sp{\rm lck}$ (lck) and an increase in p59$\sp{\rm fyn}$ (fyn). Therefore, the possibility of alterations in tyrosine

phosphorylation in anergic cells upon stimulation also was analyzed. By comparing the patterns of tyrosine phosphorylation of control vs. anergic cells upon antigen restimulation, we detected significant decreases in tyrosine phosphorylation of 38kDa (p38) and 74kDa (p74) proteins in anergic cells. Defective tyrosine phosphorylation of p38 and p74 was also detected after CD3 crosslinking in anergic cells. Defective tyrosine phosphorylation of another protein, of 34kDa (p34), was found in anergic cells after CD4 crosslinking. Both the alterations in expression levels of lck and fyn and defective tyrosine phosphorylation were reversed to normal after anergic cells recovered from nonresponsiveness by growth in exogenous IL-2. In addition, normal CD4$\sp{+}$ and CD8$\sp{+}$ T cells tolerized in vivo also showed defective tyrosine phosphorylation of p38 and p74 after CD3 crosslinking. These results demonstrate that changes in tyrosine phosphorylation dependent events correlate well with the lack of IL-2 production and may be responsible for the maintenance of nonresponsiveness in anergic T cells.

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Resistance of melanoma cell lines to interferons correlates with reduction of IFN-induced tyrosine phosphorylation. Induction of the anti-viral state by IFN is prevented by tyrosine kinase inhibitors.

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Clinical and experimental studies examining the action of IFNs on human malignant melanomas and melanoma cell lines have shown that this cancer cell type is frequently IFN resistant. In the present study, the IFN responsiveness of five melanoma cell lines, SK-MEL-28, SK-MEL-3, MM96, HT-144, and Hs 294T, as **determined** by the levels of IFN-induced expression of the antiviral proteins, 100 kDa 2',5'-oligoadenylate synthetase (OAS) and Mx Ag, was shown to correlate with the IFN responsiveness of the five lines **measured** in antiproliferative and antiviral **assays** . Three of the lines, SK-MEL-28 (IFN sensitive), SK-MEL-3 (moderately IFN sensitive), and MM96 (IFN insensitive) were analyzed further to ascertain their relative levels of IFN-activated **signal transduction** . Pretreatment of the three melanoma cell lines with the tyrosine kinase inhibitors, Herbimycin A or Genistein, produced a dose-dependent inhibition of the antiviral action of IFN-alpha, -beta, and -gamma and the induction of OAS by IFN-beta. Thus, induction of the antiviral state in melanoma cells by IFN requires activation of tyrosine kinase-dependent signaling pathways. Furthermore, the IFN responsiveness of three melanoma cell lines could be correlated with the ability to **detect** by immunoblotting of SDS-PAGE displays of cell lysates, IFN-induced tyrosine phosphorylated cellular proteins in the range m.w. 80 to 130 kDa. This induction was also sensitive to the tyrosine kinase inhibitors Herbimycin A and Genistein. Based on these results, we propose that the IFN-resistant melanoma cell lines examined contain a deficiency early in the IFN **signal transduction** pathway resulting in a reduced potential for IFN-induced tyrosine phosphorylation and a lack of responsiveness to IFN.

Tags: Human

Descriptors: *GTP-Binding Proteins; *Interferons--pharmacology--PD; *Melanoma--metabolism--ME; *Melanoma--therapy--TH; *Tyrosine--metabolism --ME; 2',5'-Oligoadenylate Synthetase--biosynthesis--BI; Drug Resistance;

Genistein; Isoflavones--pharmacology--PD; Melanoma--immunology--IM; Neoplasm Proteins--metabolism--ME; Phosphorylation; Protein-Tyrosine Kinase --antagonists and inhibitors--AI; Proteins--biosynthesis--BI; Quinones --pharmacology--PD; Semliki forest virus--immunology--IM; Tumor Cells, Cultured--drug effects--DE; Tumor Cells, Cultured--immunology--IM; Tumor Cells, Cultured--metabolism--ME; Virus Inhibitors--biosynthesis--BI
CAS Registry No.: 0 (Isoflavones); 0 (Neoplasm Proteins); 0 (Proteins); 0 (Quinones); 0 (Virus Inhibitors); 0 (protein Mx); 446-72-0 (Genistein); 55520-40-6 (Tyrosine); 70563-58-5 (herbimycin); 9008-11-1 (Interferons)
Enzyme No.: EC 2.7.1.112 (Protein- **Tyrosine Kinase**); EC 2.7.7.- (2',5'-Oligoadenylate Synthetase); EC 3.6.1.- (GTP-Binding Proteins)
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Abstract: In recent years, apoptosis, the process by which cells orchestrate their own demise, has been the subject of increasingly intense investigation, both from the stand-point of basic mechanisms of signal **transduction** and with regard to its role in **normal** and pathological processes in the nervous system, For the neurologist, an understanding of the mechanisms by which apoptosis **determines** at a cellular level the **normal** form of the nervous system, an appreciation of how both unchecked apoptosis and failure of enactment of the apoptotic pathway contribute to nervous system pathology and a sense of how both induction and inhibition of apoptosis can be exploited therapeutically are critical to applying the basic knowledge in this field to human disease, Early studies made it clear that substances produced by the target tissue influenced the survival of developing neurons, More recent investigations have demonstrated that they do so by influencing the production of a series of endogenous mediators and modulators of neuronal survival. Furthermore, it is evident that apoptosis is important for the development of both neuronal and non-neuronal cells in the peripheral and central nervous systems.
Identifiers--KeyWord Plus(R): EMBRYO SPINAL-CORD; INDUCED NEURONAL DEATH; CHICK-EMBRYO; NEUROTROPHIC FACTOR; GROWTH-FACTOR; MOTONEURON SURVIVAL; SYMPATHETIC NEURONS; MOTOR-NEURONS; RNA-SYNTHESIS; IN-VITRO
Research Fronts: 95-4914 002 (PROGRAMMED NEURONAL DEATH; APOPTOSIS IN NEURAL CELLS; BRAIN-DERIVED NEUROTROPHIC FACTOR SURVIVAL RESPONSE)
95-0477 001 (CYTOKINE RECEPTOR SIGNALING MECHANISMS; ACTIVATION OF MULTIPLE **PROTEIN - TYROSINE KINASES** ; STAT TRANSCRIPTION FACTORS; EARLY RESPONSE GENES)
95-1680 001 (PROGRAMMED CELL-DEATH; BCL-2 GENE FAMILY; REGULATION OF APOPTOSIS)
95-2493 001 (LOW-AFFINITY NEUROTROPHIN RECEPTORS; CULTURED BASAL FOREBRAIN CHOLINERGIC NEURONS; TRKA EXPRESSION; NGF TRANSGENIC MICE; NERVE GROWTH-FACTOR)

Cited References:

BARBIN G, 1984, V43, P1468, J NEUROCHEM
BARDE YA, 1982, V1, P549, EMBO J
BARRES BA, 1992, V23, P1221, J NEUROBIOL
BARRETT GL, 1994, V91, P6501, P NATL ACAD SCI USA
BREDESEN DE, 1995, V38, P839, ANN NEUROL
CAJAL SRY, 1929, P400, STUDIES VERTEBRATE N
CHUWANG IW, 1978, V177, P59, J COMP NEUROL
COWAN WM, 1973, P19, DEV AGING NERVOUS SY
COWAN WM, 1984, V225, P1258, SCIENCE
GAGLIARDINI V, 1994, V261, P826, SCIENCE
GARCIA I, 1992, V258, P302, SCIENCE
GREENLUND LJS, 1995, V14, P303, NEURON
HAMBURGER V, 1975, V160, P535, J COMP NEUROL
HAMBURGER V, 1949, V111, P457, J EXP ZOOL
HAMBURGER V, 1992, V23, P1116, J NEUROBIOL
HAMBURGER V, 1981, V1, P60, J NEUROSCI
HAMBURGER V, 1984, V4, P767, J NEUROSCI
HOLLYDAY M, 1976, V170, P311, J COMP NEUROL
HOUENOU LJ, 1995, V92, P895, P NATL ACAD SCI USA
LEVIMONTALCINI R, 1950, V86, P253, J MORPHOL
LEVIMONTALCINI R, 1960, V42, P384, P NATL ACAD SCI USA
LEVIMONTALCINI R, 1968, V48, P534, PHYSIOL REV
LIN LFH, 1993, V260, P1130, SCIENCE
MARTIN DP, 1988, V106, P829, J CELL BIOL
MARTIN DP, 1992, V23, P1205, J NEUROBIOL
MILLIGAN CE, 1994, V25, P1005, J NEUROBIOL
MILLIGAN CE, 1995, V15, P385, NEURON
NEFF NT, 1993, V24, P1578, J NEUROBIOL
OKADO N, 1984, V4, P1639, J NEUROSCI
OPPENHEIM RW, 1991, V14, P453, ANN REV NEUROSCI
OPPENHEIM RW, 1977, V125, P154, BRAIN RES
OPPENHEIM RW, 1990, V138, P104, DEV BIOL
OPPENHEIM RW, 1978, V177, P87, J COMP NEUROL
OPPENHEIM RW, 1992, V23, P1111, J NEUROBIOL
OPPENHEIM RW, 1993, V24, P1065, J NEUROBIOL
OPPENHEIM RW, 1982, V295, P57, NATURE
OPPENHEIM RW, 1992, V360, P755, NATURE
OPPENHEIM RW, 1995, V373, P344, NATURE
OPPENHEIM RW, 1988, V240, P919, SCIENCE
OPPENHEIM RW, 1991, V251, P1616, SCIENCE
OPPENHEIM RW, 1981, P74, STUDIES DEV NEUROBIO
OPPENHEIM RW, 1985, V8, P487, TRENDS NEUROSCI
PITTMAN R, 1979, V187, P425, J COMP NEUROL
PITTMAN RH, 1978, V271, P364, NATURE
SENDTNER M, 1992, V360, P757, NATURE
SNIDER WD, 1989, V26, P489, ANN NEUROL
THORNBERRY NA, 1992, V356, P768, NATURE
YAN Q, 1992, V360, P753, NATURE

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WEST Search History

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☐	L2	((modulat$ or regulat$).clm. same (brandykinin or acetylcholine or acetyl-choline or cxcr4 or cr4 or ccr5 or ccr-5 or ccr).clm. not antagon$ and antagon$)	2
☐	L3	(antagon$ not antagon$ and (screen or identify or screening or testing or identifying or library or detecting or measuring or determining).clm.)	0
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☐	L6	L5 near25 (screen$ or identif$)	427
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END OF SEARCH HISTORY

L12: Entry 1 of 11 File: USPT Jan 27, 2004

DOCUMENT-IDENTIFIER: US 6683082 B2
TITLE: Bicyclic protein kinase inhibitors

Other Reference Publication (47):
Superti-Furga et al., "A functional Screen in Yeast for Regulators and Antagonizers of Heterologous Protein Tyrosine Kinases", Nature Biotech, vol. 14:600-605, (1996).

CLAIMS:

14. The method of claim 13 wherein said serine-threonine protein kinase is selected from the group consisting of CDK2 and Raf.

DOCUMENT-IDENTIFIER: US 6689806 B1
TITLE: Indolinone compounds as kinase inhibitors

Abstract Text (1):
The invention relates to certain indolinone compounds, their method of synthesis, and a combinatorial library consisting of the indolinone compounds of the invention. The invention also relates to methods of modulating the function of protein kinases using indolinone compounds of the invention and methods of treating diseases by modulating the function of protein kinases and related signal transduction pathways.

Parent Case Text (1):
This application claims priority to U.S. Provisional Application No. 60/125,945, filed on Mar. 24, 1999, entitled "3-Arylidenyl-6-Heterocyclyl-2-indolinones as Modulators of Protein Kinase Activity," U.S. Provisional Application No. 60/127,863, filed on Apr. 5, 1999, entitled "3-Aralkyl-2-Indolinone Derivatives as Modulators of Protein Kinase Activity," U.S. Provisional Application No. 60/131,192, filed on Apr. 26, 1999, entitled "Diaryl Indolinone Compounds as Kinase Inhibitors," and U.S. Provisional No. 60/132,243, filed on May 3, 1999.

Brief Summary Text (17):
More recently, attempts have been made to identify small molecules that act as PK inhibitors. For example, bis-monocylic, bicyclic and heterocyclic aryl compounds (PCT WO 92/20642), vinylene-azaindole derivatives (PCT WO 94/14808) and 1-cyclopropyl-4-pyridylquinolones (U.S. Pat. No. 5,330,992) have been described as PTK inhibitors. Styryl compounds (U.S. Pat. No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Pat. No. 5,302,606), quinazoline derivatives (EP App. No. 0 566 266 A1), selenaindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660) and benzylphosphonic acid compounds (PCT WO 91/15495) have all been described as PTK inhibitors useful in the treatment of cancer.

Brief Summary Text (19):
The present invention is directed in part towards indolinone compounds and methods of modulating the function of protein kinases with these compounds. The methods incorporate cells that express a protein kinase. In addition, the invention describes methods of preventing and treating protein kinases-related abnormal conditions in organisms with a compound identified by the methods described herein. Furthermore, the invention pertains to pharmaceutical compositions comprising compounds identified by methods of the invention.

Brief Summary Text (20):
The present invention features indolinone compounds that potently inhibit protein kinases and related products and methods. Inhibitors of protein kinases can be obtained by adding chemical substituents to an indolinone compound. The compounds of the invention represent a new generation of therapeutics for diseases associated with one or more functional or non-functional protein kinases. Neuro-degenerative diseases and certain types of cancer fall into this class of diseases. Other diseases or disorders include dermatologic, ophthalmic, nurologic, cardiovascular, and immune disorders as well as disorders associated with abnormal angiogenesis and/or vasculogenesis. The compounds can be modified such that they are specific to their target or targets and will subsequently cause few side effects and thus represent a new generation of potential cancer therapeutics. These properties are significant improvements over the currently utilized cancer therapeutics that cause multiple side effects and deleteriously weaken patients.

Brief Summary Text (23):

Usually, indolinone compounds that are synthesized by the condensation of an oxindole compound and a ketone compound display a mixture of the possible E and Z isomers, making the isolation of the isomer of choice difficult. The compounds of the present invention feature an intermolecular hydrogen bond between the carbonyl of the oxindole compound and the hydrogen of the 1 position of the pyrrole moiety of the ketone compounds. Said hydrogen bond eliminates the problem of having a mixture of isomers by locking the intermediates in the synthesis of the indolinone compounds in the preferred conformation.

Brief Summary Text (28):
The term "indolinone" is used as that term is commonly understood in the art and includes a large subclass of substituted or unsubstituted compounds that are capable of being synthesized from an aldehyde moiety and an oxindole moiety.

Brief Summary Text (64):
A "combinatorial library" refers to all the compounds formed by the reaction of each compound of one dimension with a compound in each of the other dimensions in a multi-dimensional array of compounds. In the context of the present invention, the array is two dimensional and one dimension represents all the oxindoles of the invention and the second dimension represents all the aldehydes of the invention. Each oxindole may be reacted with each and every aldehyde in order to form an indolinone compound. All indolinone compounds formed in this way are within the scope of the present invention. Also within the scope of the present invention are smaller combinatorial libraries formed by the reaction of some of the oxindoles with all of the aldehydes, all of the oxindoles with some of the aldehydes, or some of the oxindoles with some of the aldehydes.

Brief Summary Text (92):
A. 3-Arylidenyl-6-heterocyclyl-2-indolinone Derivatives

Brief Summary Text (93):
In one aspect, the present invention relates to 3-arylidenyl-6-heterocyclyl-2-indolinone derivatives having the chemical structure set forth in formula I: ##STR3##

Brief Summary Text (107):
B. 3-Aralkyl-2-indolinone Derivatives

Brief Summary Text (108):
In another aspect, the present invention relates to 3-aralkyl-2-indolinone derivatives having the chemical structure set forth in formula II: ##STR4##

Brief Summary Text (128):
C. Diaryl Indolinone Compounds

Brief Summary Text (134):
The preferred diaryl indolinone compounds of the invention are those which are preferrably formed by the reaction of a ketone compound with and oxindole compound. The ketone compound is preferrably selected from the group consisting of ##STR6## ##STR7##

Brief Summary Text (137):
D. 4-Substituted Indolinone Compounds

Brief Summary Text (151):
B. 3-Arylidenyl-6-Heterocyclyl-2-Indolinone Derivatives

Brief Summary Text (152):
An additional aspect of this invention is a combinatorial library of at least ten 3-arylidenyl-6-heterocyclyl-2-indolinone compounds that can be formed by condensing oxindoles of structure 2 with aldehydes of structure 3.

Brief Summary Text (156):
Another aspect of this invention provides a method for the synthesis of 3-arylidenyl-6-heterocyclyl-2-indolinone of formula I comprising condensing an oxindole of formula 2 with an aldehyde of formula 3 in a solvent, preferably in the presence of a base.

Brief Summary Text (157):
Examples of the oxindoles of formula 2 that may be condensed with an aldehyde of formula 3 to give a 3-arylidenyl-6-heterocyclyl-2-indolinones of formula I are 6-(pyridin-2-yl)-2-oxindole, 6-(pyridin-3-yl)-2-oxindole, 6-(pyridine-4-yl)-2-oxindole, 6-(pyrimidin-2-yl)-2-oxindole, 6-(pyrimidin-4-yl)-2-oxindole, 6-(pyrimidin-5-yl)-2-oxindole, 6-(triazinyl)-2-oxindole, 6-(pyrrol-2-yl)-2-oxindole, 6-(pyrrol-3-yl)-2-oxindole, 6-(thiophen-2-yl)-2-oxindole, 6-(thiophen-3-yl)-2-oxindole, 6-(furan-2-yl)-2-oxindole, 6-(furan-3-yl)-2-oxindole, 6-(imidazol-2-yl)-2-oxindole, 6-(imidazol-4-yl)-2-oxindole, 6-(thiazol-2-yl)-2-oxindole, 6-(thiazol-4-yl)-2-oxindole, 6-(oxazol-2-yl)-2-oxindole, 6-(oxazol-4-yl)-2-oxindole, 6-(thiadiazol-2-yl)-2-oxindole, 6-(oxadiazol-2-yl)-2-oxindole and 6-(triazol-2-yl)-2-oxindole, 6-(3methylisoxazole-5-yl)-2-oxindole, 6-(3-methylisothiazole-5-yl)-2-oxindole, 6-(3,5-dimethyl-isoxazole-4-yl)-2-oxindole, 6-(thiazole-2-yl)-2-oxindole, 6-(thiazole-4-yl)-2-oxindole, 6-(thiazole-5-yl)-2-oxindole, 6-(3-methylthiophene-2-yl)-2-oxindole, 6-(4-methylthiophene-2-yl)-2-oxindole, 6-(5-methylthiophene-2-yl)-2-oxindole, 6-(5-chlorothiophene-2-yl)-2-oxindole, 6-(4-methylfuran-2-yl)-2-oxindole.

Brief Summary Text (159):
C. 3-Aralkyl-2-Indolinone Derivatives

Brief Summary Text (160):
Another aspect of this invention is a combinatorial library of at least ten 3-aralkyl-2-indolinone compounds that can be formed by condensing oxindoles of structure 4 with aldehydes of structure 5 and then reducing the 3-position double bond of the resultant 3-arylidene-2-oxindole. In particular, the condensation refers to reaction "A" in Scheme II. Compound 4 is the "3-arylidene-2-oxindole" referred to above.

Brief Summary Text (165):
Another aspect of this invention provides a method for the synthesis of 3-aralkyl-2-indolinone of formula II comprising condensing an oxindole of formula 4 with an aldehyde of formula 5 in a solvent, preferably in the presence of a base, optionally isolating the resultant 3-arylidene-2-oxindole and then reducing the 3-arylidene-2-oxindole.

Brief Summary Text (166):
Examples of the oxindoles of formula 4 that may be condensed with an aldehyde of formula 5 and the product reduced to give the 3-aralkyl-2-indolinones of formula II are oxindole itself and substituted oxindoles such as, without limitation, 5-fluorooxindole, 6-fluorooxindole, 7-fluorooxindole, 6-trifluoromethyloxindole, 5-chlorooxindole, 6-chlorooxindole, indole-4-carboxylic acid, 5-bromooxindole, 6-(acetamido)-oxindole, 4-methyloxindole, 5-methyloxindole, 4-methyl-5-chlorooxindole, 5-ethyloxindole, 6-hydroxyoxindole, 6-(cyclopentylcarboxamido)oxindole, 5-acetyloxindole, oxindole-5-carboxylic acid, 5-methoxyoxindole, 6-methoxyoxindole, 5-aminooxindole, 6-aminooxindole, 4-[2-(N-morpholino)ethyl]-oxindole, 7-azaoxindole, oxindole-4-carabamic acid t-

butyl ester, oxindole-6-carbamic acid t-butyl ester, 4-(2-carboxyethyl)oxindole, 4-n-butyloxindole, 4,5-dimethoxyoxindole, 6-(methanesulfonamido)oxindole, 6-(benzamido)oxindole, 5-ethoxyoxindole, 6-phenyloxindole, 4-(2-hydroxyeth-1-yl)oxindole, 6-(2-methoxyphen-1-yl)oxindole, 6-(3-methoxyphen-1-yl)oxindole and 6-(4-methoxyphen-1-yl)oxindole.

Brief Summary Text (168):
D. Diaryl Indolinone Compounds

Brief Summary Text (169):
In another aspect, the invention provides a combinatorial library of at least 10 indolinone compounds that can be formed by reacting an oxindole with a ketone, where the oxindole has the following structure ##STR21##

Brief Summary Text (179):
E. 4-Substituted Indolinone Compounds

Brief Summary Text (180):
In another aspect, the invention provides a combinatorial library of at least 10 indolinone compounds that can be formed by reacting an oxindole with an aldehyde, where the oxindole has a structure set forth in formula I, as defined herein, and where the aldehyde has the formula

Brief Summary Text (231):
Finally, the present invention is directed towards oxindole and indolinone compounds and methods of modulating the functions of protein phosphatases, as well as methods of preventing and treating protein phosphatase related abnormal conditions in organisms with a compound of the method identified above. The compounds of the invention, as well as compounds obtained by adding chemical substituents, may potently inhibit the action of phosphatases and may represent a new generation of therapeutics for diseases associated with defects in said phosphatases. Terms defined above with respect to kinases have a similar meaning to one skilled in the art with respect to phosphatases. Both RKs and non-receptor type kinases have been connected to hyperimmune disorders.

Brief Summary Text (280):
The compounds of the present invention were tested for their ability to inhibit most of protein kinase activity. The biological assays and results of these inhibition studies are reported herein. The methods used to measure modulation of protein kinase function are similar to those described in International Publication No. WO 98/07695, published Mar. 26, 1998, by Tang et al., and entitled "Indolinone Combinatorial Libraries and Related Products and Methods for the Treatment of Disease," with respect to the high throughput aspect of the method. The WO 98/07695 publication is incorporated herein by reference in its entirety, including any drawings.

Detailed Description Text (8):
A. 3-Arylidenyl-6-Heterocyclyl-2-Indolinone Derivatives

Detailed Description Text (50):
B. 3-Aralkyl-2-Indolinone Derivatives

Detailed Description Text (57):
A solution of the indolinone in methanol containing a couple of drops of acetic acid is hydrogenated over palladium on carbon overnight at room temperature. The catalyst is removed by filtration, rinsed with methanol and the filtrate concentrated to give the reduced product.

Detailed Description Text (59):
To a mixture of the indolinone (1 equiv.) in methanol and dimethylforamide is added sodium borohydride (10 equiv.). The mixture is stirred at room temperature for 1/2-3 hours. The reaction is then poured into water, extracted with ethyl acetate, washed with brine, dried and concentrated to give the reduced product.

Detailed Description Text (61):
A solution of the indolinone in methanol is hydrogenated over Pearlman's catalyst at room temperature for 1/2-10 hours. The catalyst is removed by filtration, rinsed with methanol and the filtrate concentrated to give the reduced product.

Detailed Description Text (204):
C. Diaryl Indolinone Compounds

Detailed Description Text (225):
D. 4-Substituted Indolinone Compounds

Other Reference Publication (3):
A.Aldo et al,"Synth. & Cardiotonic Activity of indolinone",Eur.J.Med.Chem.,25/2,187-90(1990).*

Other Reference Publication (6):
Andreani et al., "Synthesis and potential coanthracyclinic activity of substituted 3-(5-imidazo[2,1-b] thiazolylmethylene)-2-indolinones," Eur. J. Med. Chem. 32:919-924 (1997).

Other Reference Publication (14):
Carpenedo et al., "Identification and Measurement of Oxindole (2-Indolinone) in the Mammalian Brain and Other Rat Organs" Analytical Biochemistry 244:74-79 (1997).

Other Reference Publication (15):
Chen et al., "Effects of 3,3-Dipyridylmethyl-1-Phenyl-2-Indolinone on .gamma.-Aminobutyric Acid Elicited Chloride Current of Snail Central Neuron" Chinese Journal of Physiology 40(3):149-156 (1997).

Other Reference Publication (18):
Damiani et al., "Inhibition of Copper-Mediated Low Density Lipoprotein Peroxidation by Quinoline and Indolinone Nitroxide Radicals," Biochemical Pharmacology 48(6):1155-1161 (1994).

Other Reference Publication (19):
Davis et al., "Synthesis and microbiological properties of 3-Amino-1-Hydroxy-2-Indolinone and Related Compounds," Journal of Medicinal Chemistry 16(9):1043-1045 (1973).

Other Reference Publication (32):
Gazit et al., "Tyrphostins. 2. Heterocyclic and alpha-substituted benzylidenmalononitrile tyrphostins as potent inhibitors if EGF receptor and ErbB2-neu tyrosine kinases," J. Med. Chem. 34(6):1896-1907 (1991).

Other Reference Publication (60):
Moreto et al. "3,3-bis-(4-hydroxyphenyl)-7-methyl-2-indolinone (BHMI), the active metabolite of the laxative sulisatin" Arzneimittel-Forschung Drug Research 29(II):1561-1564 (1979).

Other Reference Publication (72):

Singh et al., "Indolinone derivatives as potential antimicrobial agents," Zentralbl. Mikrobiol. 144:105-109 (1989).

Other Reference Publication (87):
Varma and Gupta, "Nucleophilic Reactions of 2-Methyl-3-(4'-carbomethoxyphenyl)-4-quinazolinones with 2-Indolinones," J. Indian Chem. Soc. 66:804-805 (1989).

Other Reference Publication (89):
Walker, "The Reduction of Insoindogenides, Nitro Compounds, and Pyridines in a Series of 2-Indolinones," J. Med. Chem. 8(5):626-637 (1965).

Other Reference Publication (93):
Zhang et al., "Microtubule Effects of Welvistatin, a Cyanobacterial Indolinone that Circumvents Multiple Drug Resistance," Molecular Pharmacology 49:228-294 (1996).

Other Reference Publication (94):
Andreani et al., "Synthesis and potential coanthracyclinic activity of substituted 3-(5-imidazo[2,1-b] thiazolylmethylene)-2-indolinones," Eur. J. Med. Chem. 32:919-924 (1997).

CLAIMS:

6. A method for the modulation of the catalytic activity of a protein kinase comprising contacting said protein kinase with said compound of claim 1.

7. A method of modulating signal transduction pathways in cells with a compound according to claim 1, comprising the step of contacting said cells with said compound.

8. The method of claim 7, wherein said cells express a protein kinase and wherein said compound modulates the function of said protein kinase.

9. A method of identifying indolinone compounds that modulate the function of protein kinase, comprising the following steps: (a) contacting cells expressing said protein kinase with a compound of claim 1; and (b) monitoring an effect upon said cells.

10. The method of claim 9, wherein said effect is selected from the group consisting of a change in cell phenotype, a change in cell proliferation, a change in the catalytic activity of said protein kinase, and a change in the interaction between said protein kinase and a binding partner.

11. A method of regulating an unregulated protein kinase signal transduction comprising administering to a subject a therapeutically effective amount of a compound according to claim 1.

12. The method of claim 11, wherein unregulated protein kinase signal transduction leads to a cell proliferation disorder or an inflammatory disorder in an organism and said method leads to the treatment of cell proliferation disorder or inflammatory disorder; wherein said cell proliferation disorder or inflammatory disorder is associated with an aberration in a signal transduction pathway characterized by an interaction between a protein kinase and a binding partner, and wherein said method further comprises the steps of promoting or disrupting said abnormal interaction.

13. The method of claim 10, wherein said cell proliferation disorder is a cancer.

14. The method of claim 11, wherein said unregulated protein kinase signal transduction leads to a

disease that is selected from the group consisting of an immunological disorder, a hyperproliferation disorder, a cardiovascular disorder, an inflammatory disorder, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, aetherosclerosis, diabetes, and angiogenesis.

15. The method of claim 6 wherein said protein kinase is selected from the group consisting of receptor protein tyrosine kinase, cellular tyrosine kinase and serine-threonine kinase.

17. The method of claim 8 wherein said protein kinase is selected from the group consisting of receptor protein tyrosine kinase, cellular tyrosine kinase and serine-threonine kinase.

18. The method of claim 10 wherein said protein kinase is selected from the group consisting of receptor protein tyrosine kinase, cellular tyrosine kinase and serine-threonine kinase.

19. The method of claim 11 wherein said protein kinase is selected from the group consisting of receptor protein tyrosine kinase, cellular tyrosine kinase and serine-threonine kinase.

L21: Entry 64 of 67 File: USPT Sep 8, 1998

US-PAT-NO: 5804396
DOCUMENT-IDENTIFIER: US 5804396 A

TITLE: Assay for agents active in proliferative disorders

DATE-ISSUED: September 8, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Plowman; Gregory D.	San Carlos	CA		

US-CL-CURRENT: 435/7.23; 435/7.2, 436/63, 436/64

CLAIMS:

I claim:

1. A method of identifying an agent that inhibits signal transduction and may be potentially useful in treatment of cancer, comprising:

a) contacting an agent with a sample comprising a heterodimer having kinase activity, said heterodimer comprising two different components selected from the group consisting of HER2, HER3, and HER4, to render a HER2/HER3 heterodimer, a HER2/HER4 heterodimer, or a HER3/HER4 heterodimer; and

b) determining whether said agent inhibits said kinase activity, wherein inhibition of kinase activity is indicative of an agent that inhibits signal transduction.

2. The method of claim 1, wherein said heterodimer is present in a cell.

3. The method of claim 2, wherein said cell contains a recombinant nucleic acid acid encoding at least one component selected from the group consisting of said HER2, HER3, and HER4.

4. The method of claim 2, wherein said cell is selected from the group consisting of a human breast adenocarcinoma cell, a mouse fibroblast cell, and a hematopoietic cell.

5. The method of claim 1, wherein said inhibition of signal transduction is mediated by inhibition of the kinase activity of said heterodimer.

6. The method of claim 1, wherein said agent has a molecular weight of less than 3000.

7. The method of claim 1, wherein said agent is selected from the group consisting of quinazolines, tyrphostins and quinoxalines.

8. The method of claim 1, wherein said agent inhibits the stimulation of signal transduction by a ligand selected from the group consisting of gp30, neu neu differentiation factor, heregulin, and Beta-cellulin which is added to

said sample, wherein said ligand is able to mediate the kinase activity of said said HER2/HER3 heterodimer, said HER3/HER4 heterodimer, or said HER2/HER4 heterodimer.

9. The method of claim 8, wherein said ligand is beta-cellulin or heregulin.

10. The method of claim 8, wherein said agent is contacted with said sample prior to addition of said ligand.

11. The method of claim 1, wherein said determining step comprises measuring the level of phosphorylation of at least one of said HER2, said HER3 or said HER4.

12. A method of screening for an agent that specifically inhibits the kinase activity of a HER2 or HER4 receptor tyrosine kinase and may be potentially useful in treatment of cancer, comprising:

a) contacting an agent with a sample comprising a HER2/HER3, HER2/HER4, or HER3/HER4 heterodimer having kinase activity, said heterodimer comprising a catalytically active receptor tyrosine kinase selected from the group consisting of HER2 and HER4 conjugated to a catalytically inactive receptor tyrosine kinase selected from the group consisting of HER 2, HER3 and HER4; and and

b) determining whether said agent inhibits the kinase activity of said heterodimer, wherein inhibition of kinase activity is indicative of an agent that specifically inhibits the kinase activity of a HER2 or HER4 receptor tyrosine kinase.

13. The method of claim 12, wherein said heterodimer is a HER2/HER4 heterodimer heterodimer and said catalytically inactive receptor tyrosine kinase is HER2.

14. The method of claim 12, wherein said heterodimer is a HER2/HER4 heterodimer heterodimer and said catalytically inactive receptor tyrosine kinase is HER4.

15. A method of screening for an agent that specifically inhibits the kinase activity of a HER2 component or a HER4 component of a HER2/HER4 heterodimer and may be potentially useful in treatment of cancer, comprising

a) contacting an agent with a sample comprising a HER2/HER4 heterodimer comprising a HER2 component and a HER4 component, wherein one of said HER2 and HER4 components lacks kinase activity; and

b) determining whether said agent inhibits the kinase activity of said heterodimer, wherein inhibition of kinase activity is indicative of an agent that specifically inhibits the kinase activity of a HER2 or HER4 component.

16. A method of screening for an agent that specifically inhibits the kinase activity of a HER2 or HER4 receptor tyrosine kinase and may be potentially useful in treatment of cancer, comprising:

a) contacting an agent with a cell comprising a heterodimer to form a first sample, wherein said heterodimer comprises i) a first receptor tyrosine kinase component that possesses kinase activity, said first receptor tyrosine kinase component selected from the group consisting of HER2 and HER4 and associated with ii) a second receptor tyrosine kinase component that lacks kinase activity, said second tyrosine component selected from the group consisting of

HER2, HER3 or HER4;

b) determining the level of signal transduction by measuring kinase activity or or cell growth in said cell in said first sample;

c) adding a ligand selected from the group consisting of gp30, neu differentiation factor, heregulin, and Beta-cellulin that stimulates the kinase activity of said heterodimer to said first sample, thereby forming a second sample;

e) determining the level of signal transduction by measuring kinase activity or or cell growth in said second sample; and

f) comparing the levels of signal transduction in said first sample and said second sample, thereby determining whether said agent inhibits the kinase activity of a HER2 or HER4 receptor tyrosine kinase.

17. The method of claim 16, wherein said steps c) and e) comprise measuring HER2 kinase activity.

18. The method of claim 16, wherein said steps c) and e) comprise measuring phosphorylation of HER2 or a HER2 substrate.

US005804396A

United States Patent [19]

Plowman

[11] Patent Number: 5,804,396

[45] Date of Patent: Sep. 8, 1998

[54] **ASSAY FOR AGENTS ACTIVE IN PROLIFERATIVE DISORDERS**

[75] Inventor: **Gregory D. Plowman**, San Carlos, Calif.

[73] Assignee: **Sugen, Inc.**, Redwood City, Calif.

[21] Appl. No.: 322,868

[22] Filed: Oct. 12, 1994

[51] Int. Cl.[6] G01N 33/574; G01N 33/53; G01N 33/567; G01N 33/48

[52] U.S. Cl. 435/7.23; 435/7.2; 436/63; 436/64

[58] Field of Search 435/7.2, 7.23; 436/63, 64

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,343,940	8/1982	Kreighbaum et al.	544/283
4,447,608	5/1984	Jones et al.	544/287
4,757,072	7/1988	Kabbe et al.	514/257
5,316,553	5/1994	Kaul et al.	8/639

FOREIGN PATENT DOCUMENTS

0520722	12/1992	European Pat. Off. .
0562734	9/1993	European Pat. Off. .
9220642	11/1992	WIPO .

OTHER PUBLICATIONS

Gura. T. "Systems for identifying new drugs are often faulty" Science. vol. 278. pp. 1041–1042, Nov. 7, 1997.

Dermer G. "Another Anniversary for the war on Cancer" Bio/Technology. vol. 12. p. 320, Mar. 1994.

King et al, "High throughput assay for inhibitors of the epidermal growth factor receptor–associated tyrosine kinase" Life Sciences, vol. 53, pp. 1465–1472, 1993.

Aaronson, "Growth Factors and Cancer," *Science*, 254:1146–1153 (1991).

Allen et al., "Modulation of CD4 by Suramin," *Clin. Exp. Immunol.* 91:141–156 (1993).

Anafi et al., "Tyrphostin–Induced Inhibition of $p210^{bcr-abl}$ Tyrosine Kinase Activity Induces K562 to Differentiate," *Blood* 82:3524–3529 (1993).

Andrews et al., "Report of the AVMA Panel on Euthanasia," *Journal of American Veterinary Medical Assoc.* 202:229–249 (1993).

Bacus et al., "A Ligand for the erbB–2 Oncongene Product (gp30) Induces Differentiation of Human Breast Cancer Cells," *Cell Growth and Diff.* 3:401–411 (1992).

Baker et al., "Induction of Acetylcholine Receptor Clustering By Native Polystyrene Beads: Implication of an endogenous muscle–derived signalling system," *J. Cell Sci.* 102:543–555 (1992).

Barker et al., "In vitro activity of non–glutamate containing quinazoline–based thymidylate synthase inhibitors," *Proc. of Am. Assoc. for Cancer Research* 32:327 (1991).

Bertino, "Toward Improved Selectivity in Cancer Chemotherapy: The Richard and Hinda Rosenthal Foundation Award Lecture," *Cancer Research* 39:293–304 (1979).

Bilder et al., "Tyrphostins inhibit PDGF–induced DNA synthesis and associated early events in smooth muscle cells," *Am. J. Physiol.* 260(Cell Physiol.29):C721–C730 (1991).

Brunton et al., "Anti–Tumor Activity of Novel Tyrphostins in Breast Cancer Cells," *Proceedings of Amer. Assoc. Cancer Rsch.* 33:558 (1992).

Bryckaert et al., "Inhibition of Platelet–Derived Growth Factor–Induced Mitogenesis and Tyrosine Kinase Activity in Cultured Bone Marrow Fibroblasts by Tyrphostins," *Exp. Cell Research*, 199:255–261 (1992).

Burke et al., "Bicyclic Compounds as Ring–Constrained Inhibitors of Protein Tyrosine Kinase $p56^{lck}$ [1]," *J. Med. Chem.* 36:425–432 (1993).

Burke et al., "Arylamides of Hydroxylated Isoquinolines as Protein–Tyrosine Kinase Inhibitors," *BioOrganic Med. Chem. Letters* 2:1771–1774 (1992).

Caraglia et al., "Cytosine Arabinoside Increases the Binding of ^{125}I–Labelled Epidermal Growth Factor and ^{125}I–Transferrin And Enhances the In Vitro Targeting of Human Tumour Cells With Anti–(Growth Factor Receptor)mAb," *Cancer Immunol. Immunother.* 37:150–156 (1993).

Caraway and Cantley, "A Neu Acquaintance for ErbB3 and ErbB4: A Role for Receptor Hereodimerization in Growth Signaling", *Cell* 78:5–8 (1994).

Carraway et al., "The erbB3 Gene Product Is a Receptor for Heregulin", *J. Biol. Chem.* 269:14303–14306 (1994).

Chou and Haman, "Characterization of a member of the immunoglobulin gene superfamily that possibly represents an additional class of growth factor receptor," *Proc. Natl. Acad. Sci. USA* 88:4897–4901 (1991).

Curtin et al., "Inhibition of the growth of human hepatocellular carcinoma in vitro and in athymic mice by a quinazoline inhibitor of thymidylate synthase, CB3717," *Br. J. Cancer* 53:361–368 (1986).

Culouscou et al., "Characterization of a Breast Cancer Cell Differentiation Factor That Specifically Activates the HER4/$p180^{erbB4}$ Receptor", *J. Biol. Chem.* 268:18407–18410 (1993).

Dati et al., "Inhibition of c–erbB–2 oncogene expression by estrogens in human breast cancer cells", *Oncogene* 5:1001–1006 (1990).

Decker and Lohmann–Matthes, "A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity," *J. Immunol. Methods*, 115:61–69 (1988).

(List continued on next page.)

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[57] **ABSTRACT**

Method for identifying an agent for treatment of a proliferative disorder, comprising the steps of assaying a potential agent for activity in inhibition of signal transduction by a HER2/HER3 or HER2/HER4 or HER3/HER4 heterodimer. Method of screening for an agent that selectively inhibits the kinase activity of a heterodimer having one catalytically inactive component.

18 Claims, No Drawings

DOCUMENT-IDENTIFIER: US 6300098 B1
TITLE: Human signal transduction serine/threonine kinase

Brief Summary Text (13):
Integral members of cellular signaling pathways as targets for therapeutic development, for example, have been the subject to several reviews. See, e.g., Levitzki, A., Signal-Transduction Therapy: A Novel Approach to Disease Management, Eur. J. Biochem, 226:1 (1994); Powis G., The Potentialfor Molecular Oncology to Define New Drug Targets, in: New Molecular Targets for Cancer Chemotherapy, Workman, P., Kerr D. J., eds., CRC Press, Boca Raton FL (1994). As a result of the efforts of numerous laboratories, an impressive list of remarkably specific inhibitors of kinases, for instance, has become available. See, e.g., Levitzki, A., Tyrphostins: Tyrosine Kinase Blockers as Novel Antiproliferative Agents and Dissectors of Signal Transduction, FASEB; 6:3275 (1992); Workman P., et al., Discovery and Design of Inhibitors of Oncogenic Tyrosine Kinases, in: New Approaches in Cancer Pharmacology: Drug Design and Development, Springer, Berlin 55 (1994).

CLAIMS:

2. A method of identifying compounds that modulate a biological and/or pharmacological activity of a signal transduction kinase polypeptide, comprising:

(a) combining a candidate compound modulator with a polypeptide according to claim 1, and

(b) measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide.

3. A method of identifying compounds that modulate a biological and/or pharmacological activity of a signal transduction kinase polypeptide according to claim 2, comprising:

(a) combining a candidate compound modulator with a host-cell which expresses said polypeptide, and

(b) measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide.

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PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020183364 A1

TITLE: Methods of modulating protein tyrosine kinase function with substituted indolinone compounds

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tang, Peng Cho	Moraga	CA	US	

ASSIGNEE-INFORMATION:

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SUGEN, Inc.				02

APPL-NO: 10/ 013944 [PALM]
DATE FILED: December 13, 2001

RELATED-US-APPL-DATA:
Application 10/013944 is a continuation-of US application 09/407164, filed September 28, 1999, PENDING
Application is a non-provisional-of-provisional application 60/102178, filed September 28, 1998,

INT-CL: [07] A61 K 31/4439, A61 K 31/404, C07 D 43/14, C07 D 43/02

US-CL-PUBLISHED: 514/339; 514/414, 548/455, 546/277.4
US-CL-CURRENT: 514/339; 514/414, 546/277.4, 548/455

ABSTRACT:

The invention relates to certain indolinone compounds, their method of synthesis, and a combinatorial library consisting of the indolinone compounds. The invention also relates to methods of modulating the function of protein kinases using indolinone compounds and methods of treating diseases by modulating the function of protein kinases and related signal transduction pathways.

RELATED APPLICATIONS

[0001] This application is related to the U.S. Patent Applications Serial No. 60/102,178, filed Sep. 28, 1998, Peng C. Tang, and entitled "METHODS OF MODULATING TYROSINE KINASE FUNCTION WITH SUBSTITUTED INDOLINONE COMPOUNDS" (Lyon & Lyon Docket No. 236/137), and Ser. No. 08/915,366, filed Aug. 20, 1997, by Tang et al., and entitled "INDOLINONE COMBINATORIAL LIBARIES AND RELATED PRODUCTS AND METHODS FOR THE TREATMENT OF DISEASE" (Lyon & Lyon Docket No. 227/111), and Ser. No. 09/129,256, filed Aug. 4, 1998, by Tang et al., and entitled "METHODS OF MODULATING PROTEIN TYROSINE KINASE FUNCTION WITH SUBSTITUTED INDOLINONE COMPOUNDS" (Lyon & Lyon Docket No. 230/177), which are hereby incorporated by reference herein in their entirety, including any drawings.

Entry 45 of 67 File: PGPB Aug 16, 2001

PGPUB-DOCUMENT-NUMBER: 20010014679
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010014679 A1

TITLE: Methods of modulating serine/threonine protein kinase function with quinazoline-based compounds

PUBLICATION-DATE: August 16, 2001

INVENTOR-INFORMATION:

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Kutscher, Bernhard	Maintal		DE	
App, Harald	San Francisco		US	

US-CL-CURRENT: 514/252.17; 514/266.2, 514/266.21, 514/266.31

CLAIMS:

What is claimed is:

1. A method of modulating the function of a serine/threonine protein kinase with a quinazoline-based compound substituted at the 5-position with an optionally substituted five-membered or six-membered aryl or heteroaryl ring, comprising the step of contacting cells expressing said serine/threonine protein kinase with said compound.

2. The method of claim 1, wherein said serine/threonine protein kinase is RAF.

3. A method of identifying compounds that modulate the function of serine/threonine protein kinase, comprising the following steps: (a) contacting cells expressing said serine/threonine protein kinase with said compound; and (b) monitoring an effect upon said cells.

4. The method of claim 3, wherein said effect is a change or an absence of a change in cell phenotype.

5. The method of claim 3, wherein said effect is a change or an absence of a change in cell proliferation.

6. The method of claim 3, wherein said effect is a change or absence of a change in the catalytic activity of the said serine/threonine protein kinase.

7. The method of claim 3, wherein said effect is a change or absence of a change in the interaction between said serine/threonine protein kinase with a natural binding partner, as described herein.

8. The method of claim 3, comprising the following steps: (a) lysing said cells to render a lysate comprising serine/threonine protein kinase; (b) adsorbing said serine/threonine protein kinase to an

antibody; (c) incubating said adsorbed serine/threonine protein kinase with a substrate or substrates; and (d) adsorbing said substrate or substrates to a solid support or antibody; wherein said step of monitoring said effect on said cells comprises measuring the phosphate concentration of said substrate or substrates.

9. The method of claim 3, wherein said serine/threonine protein kinase is RAF and comprises the following steps: (a) lysing said cells to render a lysate comprising RAF; (b) adsorbing said RAF to an antibody; (c) incubating the adsorbed RAF with MEK and MAPK; and (d) adsorbing said MEK and MAPK to a solid support or antibody or antibodies; wherein said step of measuring said effect on said cells comprises monitoring the phosphate concentration of said MEK and MAPK.

10. The method of claim 1, wherein said quinazoline-based compound has the formula set forth in structure I, II, or III: 16wherein (a) Z is oxygen, NX.sub.1, or sulfur, where X.sub.1 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (b) n is 0, 1, 2, 3, or 4; (c) A.sub.1, A.sub.2, A.sub.3, A.sub.4, A.sub.5, and A.sub.6 are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; (d) R.sub.1, R.sub.2, R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (iv) halogen or trihalomethyl; (v) a ketone of formula --CO--X.sub.4, where X.sub.4 is selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl moieties; (vi) a carboxylic acid of formula --(X.sub.5).sub.n--COOH or ester of formula --(X.sub.6).sub.n--COO--X.sub- .7, where X.sub.5, X.sub.6, and X.sub.7 and are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl moieties and where n is 0 or 1; (vii) an alcohol of formula (X.sub.8).sub.n--OH or an alkoxy moiety of formula --(X.sub.8).sub.n--O--X.sub.9, where X.sub.8 and X.sub.9 are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl ring moieties and where n is 0 or 1, and where said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (viii) 'NHCOX.sub.10, where X.sub.10 is selected from the group consisting of alkyl, hydroxyl, and five-membered or six-membered aryl or heteroaryl ring moieties, wherein said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (ix) --SO.sub.2NX.sub.11X.sub.12, where X.sub.11 and X.sub.12 are selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (x) a five-membered or six-membered aryl or heteroaryl ring moiety optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester moieties; (g) any adjacent R.sub.3, R.sub.4, and R.sub.5 or any adjacent R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are fused together to form a five-membered or six-membered aryl or heteroaryl ring moiety, wherein said five-membered or six-membered aryl or heteroaryl ring comprises two carbon atoms of the quinazoline ring; (h) R.sub.11 and R.sub.12 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; and (i) Z' is carbon, oxygen, sulfur, or nitrogen and R.sub.13 and R.sub.14 taken together form a five-membered or six-membered heteroaryl ring with Z' as a ring member.

11. The method of claim 1, wherein said quinazoline-based compound has the formula set forth in structure I, II, or III: 17wherein (a) Z is oxygen, NX.sub.1, or sulfur, where X.sub.1 is selected from the group consisting of hydrogen and saturated or unsaturated alkyl; (b) n is 0, 1, or 2; (c) A.sub.1, A.sub.2, A.sub.3, A.sub.4, A.sub.5, and A.sub.6 are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; (d) R.sub.1 and R.sub.2 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen and saturated or unsaturated

alkyl; and (iv) halogen or trihalomethyl; (v) five-membered or six-membered aryl or heteroaryl ring moiety; (e) R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen or trihalomethyl; (v) C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety; (f) any adjacent R.sub.3, R.sub.4, and R.sub.5 or any adjacent R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are fused together to form a five-membered or six-membered aryl or heteroaryl ring moiety, wherein said five-membered or six-membered aryl or heteroaryl ring comprises two carbon atoms of the quinazoline ring; (g) R.sub.11 and R.sub.12 are independently selected from the group consisting of (i) hydrogen; and (ii) saturated or unsaturated alkyl; and (h) Z' is nitrogen, oxygen, or sulfur and R.sub.13 and R.sub.14 taken together form a five-membered or six-membered heteroaryl ring moiety with Z' as a ring member, wherein said ring is optionally substituted with one, two, or three alkyl, halogen, trihalomethyl, carboxylate, and ester moieties.

12. The method of claim 1, wherein said quinazoline-based compound has the structure set forth in formula IV or V: 18wherein (a) Z is oxygen or sulfur; (b) n is 0 or 1; (c) R.sub.1 and R.sub.2 are independently selected f rom the group consisting of (i) hydrogen; and (ii) NX.sub.1X.sub.2 where X.sub.1 and X.sub.2 independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; (iii) benzyl; (d) R.sub.3, R.sub.4, and R.sub.5 are independently selected from the group consisting of (i) hydrogen; and (ii) saturated or unsaturated alkyl; (iii) NX.sub.3X.sub.4, where X.sub.3 and X.sub.4 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; (e) R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.5X.sub.6, where X.sub.5 and X.sub.6 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen or trihalomethyl; (v) C (X.sub.7) .sub.3, where X.sub.7 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) methoxy; (f) R.sub.11 and R.sub.12 are hydrogen; and (g) Z' is nitrogen and R.sub.13 and R.sub.14 taken together form a five-membered heteroaryl ring.

13. The method of claim 1, wherein said quinazoline-based compound has a structure set forth in formula VI or VII: 19wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

14. The method of claim 1, wherein said quinazoline-based compound has a structure set forth in formula VIII or IX: 20wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of

hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

15. The method of claim 1, wherein said quinazoline-based compound has a structure set forth in formula X: (X): 21wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, and R.sub.6 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C (X.sub.6) .sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

16. The method of claim 1, wherein said quinazoline-based compound is selected from the group consisting of: 22

17. A method of preventing or treating an abnormal condition in an organism, comprising the step of administering a quinazoline-based compound of formula I, II, or III to said organism: 23wherein (a) Z is oxygen, NX.sub.1, or sulfur, where X.sub.1 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (b) n is 0, 1, 2, 3, or 4; (c) A.sub.1, A.sub.2, A.sub.3, A.sub.4, A.sub.5, and A.sub.6 are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; (d) R.sub.1, R.sub.2, R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (iv) halogen or trihalomethyl; (v) a ketone of formula --CO--X.sub.4, where X.sub.4 is selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl moieties; (vi) a carboxylic acid of formula --(X.sub.5).sub.n--COOH or ester of formula --(X.sub.6).sub.n--COO--X.sub- .7, where X.sub.5, X.sub.6, and X.sub.7 and are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl moieties and where n is 0 or 1; (vii) an alcohol of formula (X.sub.8).sub.n--OH or an alkoxy moiety of formula --(X.sub.8).sub.n--O--X.sub.9, where X.sub.8 and X.sub.9 are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl ring moieties and where n is 0 or 1, and where said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (viii) --NHCOX.sub.10, where X.sub.10 is selected from the group consisting of alkyl, hydroxyl, and five-membered or six-membered aryl or heteroaryl ring moieties, wherein said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (ix) --SO.sub.2NX.sub.11X.sub.12, where X.sub.11 and X.sub.12 are selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (x) a five-membered or six-membered aryl or heteroaryl ring moiety optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester moieties; (e) any adjacent R.sub.3, R.sub.4, and R.sub.5 or any adjacent R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are fused together to form a five-membered or six-membered aryl or heteroaryl ring moiety, wherein said five-membered or six-membered aryl or heteroaryl ring comprises two carbon atoms of the quinazoline ring; (f) R.sub.11 and R.sub.12 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (g) Z' is carbon, oxygen, sulfur, or nitrogen and R.sub.13 and R.sub.14 taken together form a five-membered or six-membered heteroaryl ring with Z' as a ring member.

18. The method of claim 17, wherein said quinazoline-based compound has a structure set forth in formula VI or VII: 24where (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C (X.sub.6) .sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

19. The method of claim 17, wherein said quinazoline-based compound has a structure set forth in formula VIII or IX: 25where (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

20. The method of claim 17, wherein said quinazoline-based compound has a structure set forth in formula X: 26wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, and R.sub.6 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

21. The method of claim 17, wherein said organism is a mammal.

22. The method of claim 17, wherein said abnormal condition is cancer or a fibrotic disorder.

23. The method of claim 22, wherein said abnormal condition is a cancer selected from the group consisting of lung cancer, ovarian cancer, breast cancer, brain cancer, intra-axial brain cancer, colon cancer, prostate cancer, Kaposi's sarcoma, melanoma, and glioma.

24. The method of claim 17, wherein said abnormal condition is associated with an aberration in a signal transduction pathway characterized by an interaction between a serine/threonine protein kinase and a natural binding partner.

25. The method of claim 24, wherein said serine/threonine protein kinase is RAF.

26. A quinazoline compound having a structure set forth in formula I, II, or III: 27wherein (i) Z is oxygen, NX.sub.1, or sulfur, where X.sub.1 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (iii) A.sub.1, A.sub.2, A.sub.3, A.sub.4, A.sub.5, and A.sub.6 are independently selected from the group consisting of

carbon, nitrogen, oxygen, and sulfur; (iv) R.sub.1 and R.sub.2 are independently selected from the group consisting of (a) hydrogen; (b) saturated or unsaturated alkyl; (c) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (d) halogen or trihalomethyl; (e) five-membered or six-membered aryl or heteroaryl ring moiety; (v) R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of: (a) hydrogen, provided that at least one of R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 is a non-hydrogen moiety if R.sub.2 is --NH.sub.2; (b) saturated or unsaturated alkyl, wherein said R.sub.8 is not methyl when R.sub.2 is --NH.sub.2 and when n=1; (c) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (d) halogen or trihalomethyl, wherein said R.sub.8 is not chlorine or fluorine when R.sub.2 is --NH.sub.2 and when n=1; (e) a ketone of formula --CO--X.sub.4, where X.sub.4 is selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl moieties; (f) a carboxylic acid of formula --(X.sub.6).sub.n--COOH or ester of formula --(X.sub.6).sub.n--COO--X.sub.7, where X.sub.5, X.sub.6, and X.sub.7 and are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl moieties and where n is 0 or 1; (g) an alcohol of formula (X.sub.8).sub.n--OH or an alkoxy moiety of formula --(X.sub.8).sub.n--O--X.sub.9, where X.sub.8 and X.sub.9 are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl ring moieties and where n is 0 or 1, and wherein said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (h) --NHCOX.sub.10, where X.sub.10 is selected from the group consisting of alkyl, hydroxyl, and five-membered or six-membered aryl or heteroaryl ring moieties, wherein said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (i) --SO.sub.2NX.sub.11X.sub.12, where X.sub.11 and X.sub.12 are selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (j) a five-membered or six-membered aryl or heteroaryl ring moiety optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester moieties; (v) any adjacent R.sub.3, R.sub.4, and R.sub.5 or any adjacent R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are fused together to form a five-membered or six-membered aryl or heteroaryl ring moiety, wherein said five-membered or six-membered aryl or heteroaryl ring comprises two carbon atoms of the quinazoline ring; (vi) R.sub.11 and R.sub.12 are independently selected from the group consisting of (a) hydrogen; (b) saturated or unsaturated alkyl; and (vii) Z' is carbon, oxygen, sulfur, or nitrogen and R.sub.13 and R.sub.14 taken together form a five-membered or six-membered heteroaryl ring with Z' as a ring member.

27. A quinazoline compound having the structure set forth in formula I, II, or III: 28wherein (a) Z is oxygen, NX.sub.1, or sulfur, where X.sub.1 is selected from the group consisting of hydrogen and saturated or unsaturated alkyl; (b) n is 0, 1, or 2; (c) A.sub.1, A.sub.2, A.sub.3, A.sub.4, A.sub.5, and A.sub.6 are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; (d) R.sub.1 and R.sub.2 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen or trihalomethyl; (v) five-membered or six-membered aryl or heteroaryl ring moiety; (e) R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of: (i) hydrogen, provided that at least one of R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 is a non-hydrogen moiety if R.sub.2 is --NH.sub.2; (ii) saturated or unsaturated alkyl, wherein said R.sub.8 is not methyl when R.sub.2 is --NH.sub.2 and when n=1; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen or trihalomethyl, wherein said R.sub.8 is not chlorine or fluorine when R.sub.2 is --NH.sub.2 and when n=1; (v) C(X.sub.6).sub.3, where X.sub.6

is selected from the group consisting of fluorine, chlorine, bromine, and iodine; (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety; (f) any adjacent R.sub.3, R.sub.4, and R.sub.5 or any adjacent R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are fused together to form a five-membered or six-membered aryl or heteroaryl ring moiety, wherein said five-membered or six-membered aryl or heteroaryl ring comprises two carbon atoms of the quinazoline ring; (g) R.sub.11 and R.sub.12 are independently selected from the group consisting of (i) hydrogen; and (ii) saturated or unsaturated alkyl; and (h) Z' is nitrogen, oxygen, or sulfur and R.sub.13 and R.sub.14 taken together form a five-membered or six-membered heteroaryl ring moiety with Z' as a ring member, wherein said ring is optionally substituted with one, two, or three alkyl, halogen, trihalomethyl, carboxylate, and ester moieties.

28. A quinazoline compound having the structure set forth in formula IV or V: 29wherein (a) Z is oxygen or sulfur; (b) n is 0 or 1; (c) R.sub.1 and R.sub.2 are independently selected from the group consisting of (i) hydrogen; and (ii) NX.sub.1X.sub.2, where X.sub.1 and X.sub.2 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; (iii) benzyl; (d) R.sub.3, R.sub.4, and R.sub.5 are independently selected from the group consisting of (i) hydrogen; and (ii) saturated or unsaturated alkyl; (iii) NX.sub.3X.sub.4, where X.sub.3 and X.sub.4 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; (e) R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 are independently selected from the group consisting of: (i) hydrogen, provided that at least one of R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 is a non-hydrogen moiety if R.sub.2 is --NH.sub.2; (ii) saturated or unsaturated alkyl, wherein said R.sub.8 is not methyl when R.sub.2 is --NH.sub.2 and when n=1; (iii) NX.sub.5X.sub.6, where X.sub.5and X.sub.6 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen or trihalomethyl, wherein said R.sub.8 is not chorine or fluorine when R.sub.2 is --NH.sub.2 and when n=1 (v) C (X.sub.7).sub.3, where X.sub.7 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) methoxy; (f) R.sub.11 and R.sub.12 are hydrogen; and (g) Z' is nitrogen and R.sub.13 and R.sub.14 taken together form a five-membered heteroaryl ring.

29. A quinazoline compound having a structure set forth in formula VI or VII: 30wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 are independently selected from the group consisting of (i) hydrogen, provided that at least one of R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 is a non-hydrogen moiety if R.sub.2 is --NH.sub.2; (ii) saturated or unsaturated alkyl, wherein said R.sub.5 is not methyl when R.sub.2 is --NH.sub.2; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen, wherein said R.sub.5 is not chlorine or fluorine when R.sub.2 is --NH.sub.2; (v) C (X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

30. A quinazoline compound having a structure set forth in formula VIII or IX: 31wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, R.sub.6, and R.sub.7 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-

membered or six-membered aryl or heteroaryl ring moiety.

31. A quinazoline compound having a structure set forth in formula X: 32wherein (a) R.sub.1 and R.sub.2 are independently selected from the group consisting of hydrogen and --NH.sub.2, provided at least one of R.sub.1 and R.sub.2 is --NH.sub.2; (b) R.sub.3, R.sub.4, R.sub.5, and R.sub.6 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.4X.sub.5, where X.sub.4 and X.sub.5 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen; (v) C(X.sub.6).sub.3, where X.sub.6 is selected from the group consisting of fluorine, chlorine, bromine, and iodine; and (vi) OX.sub.7, where X.sub.7 is selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and a five-membered or six-membered aryl or heteroaryl ring moiety.

32. A quinazoline compound selected from the group consisting of: 33

33. A pharmaceutical composition comprising a quinazoline compound of any one of claims 26-32 or salt thereof, and a physiologically acceptable carrier or diluent.

34. A method for synthesizing a compound of claim 26, comprising the steps of: (a) reacting a first reactant with a second reactant to yield said compound, wherein said first reactant has a structure of formula XI: 34and wherein said second structure has a structure of formula (XII) or (XIII): 35wherein, (a) Z is oxygen or sulfur; (b) n is 0, 1, 2, 3, or 4; (c) A.sub.1, A.sub.2, A.sub.3, A.sub.4, A.sub.5, and A.sub.6 are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; (d) R.sub.1 and R.sub.2 are independently selected from the group consisting of (i) hydrogen; (ii) saturated or unsaturated alkyl; (iii) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen and saturated or unsaturated alkyl; and (iv) halogen or trihalomethyl; (v) five-membered or six-membered aryl or heteroaryl ring moiety; (e) R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9,and R.sub.10 are independently selected from the group consisting of: (i) hydrogen, provided that at least one of R.sub.3, R.sub.4, R.sub.5, R.sub.6, R.sub.7, R.sub.8, R.sub.9, and R.sub.10 is a non-hydrogen moiety if R.sub.2 is --NH.sub.2; (ii) saturated or unsaturated alkyl, wherein said R.sub.6 is not methyl when R.sub.2 is --NH.sub.2 and when n=1; (iii) NX.sub.2X.sub.3, where X.sub.2 and X.sub.3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl ring moieties; (iv) halogen or trihalomethyl, wherein said R.sub.8 is not chlorine or fluorine when R.sub.2 is --NH.sub.2 and when n=1; (v) a ketone of formula --CO--X.sub.4, where X.sub.4 is selected from the group consisting of hydrogen, alkyl, and five-membered or six-membered aryl or heteroaryl moieties; (vi) a carboxylic acid of formula --(X.sub.5).sub.n--COOH or ester of formula --(X.sub.6).sub.n--COO--X.sub.7, where X.sub.5, X.sub.6, and X.sub.7 and are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl moieties and where n is 0 or 1; (vii) an alcohol of formula (X.sub.8).sub.n--OH or an alkoxy moiety of formula --(X.sub.8).sub.n--O--X.sub.9, where X.sub.8 and X.sub.9 are independently selected from the group consisting of alkyl and five-membered or six-membered aryl or heteroaryl ring moieties and where n is 0 or 1, and wherein said ring moieties are optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, and ester; (viii) --NHCOX.sub.10, where X.sub.10 is selected from the group consisting of alkyl, hydroxyl, and five-membered or six-membered aryl or heteroaryl ring moieties, wherein said ring moieties are optionally substituted with

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L7: Entry 8 of 12 File: DWPI Dec 29, 1998

DERWENT-ACC-NO: 1999-094912
DERWENT-WEEK: 200221

TITLE: Mitogen activated protein kinase kinases and their fragments - used for regulating signalling from growth factor receptors, e.g. to modulate apoptosis for treatment of cancer, autoimmune disease and inflammation

Basic Abstract Text (6):
USE - MEKKs phosphorylate and activate MEK proteins and other signal transduction molecules, so can regulate signalling initiated from a growth factor receptor in a way different from that involving Raf protein. Particularly MEKKs, or their fragments, are involved in regulation of apoptosis so they, or agents that increase their activity, are used to treat cancers, autoimmune diseases, inflammation, allergies, neuronal disorders (e.g. Alzheimer's or Parkinson's diseases) and in wound healing. MEKKs are also useful for identifying agents (A) that regulate signal transduction from cell surface receptors (e.g. from their effect on ability of MEKK to phosphorylate a substrate such as MEK or Jun extracellular signal-regulated kinase).

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L13: Entry 3 of 6 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019005
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020019005 A1

TITLE: Process for identification of genes encoding proteins having cell proliferation-promoting activity

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kamb, Carl Alexander	Salt Lake City	UT	US	

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	COUNTRY	TYPE CODE
Arcaris, Inc.				02

APPL-NO: 09/ 921101 [PALM]
DATE FILED: August 2, 2001

RELATED-US-APPL-DATA:
Application 09/921101 is a continuation-of US application 09/252204, filed February 18, 1999, PENDING

INT-CL: [07] C12 Q 1/68, G01 N 33/53

US-CL-PUBLISHED: 435/6; 435/7.1
US-CL-CURRENT: 435/6; 435/7.1

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention is directed to selection systems for the identification of cell proliferation genes based on functional analysis. More specifically, the invention is directed to a process for the identification of a cell proliferation promoting activity, the isolation of genes involved in such cell proliferation promoting activity, and the use of the so identified genes for the diagnosis or treatment of a disease associated with excessive cell proliferation. The invention further is directed to the design and development of antibodies, peptides, nucleic acids, and other compounds which specifically interfere with the function of the identified gene and/or its gene product, and pharmaceutical compositions comprising such compounds, for the treatment of diseases associated with inappropriate or unregulated cell proliferation.

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L13: Entry 3 of 6 File: PGPB Feb 14, 2002

DOCUMENT-IDENTIFIER: US 20020019005 A1
TITLE: Process for identification of genes encoding proteins having cell proliferation-promoting activity

CLAIMS:

8. The process of claim 3, wherein said gene is a dominant negative oncogene selected from the group consisting of cJUN, EGF-R, GRB2, RAF, A, RAS, SRC, and tyrosine kinase receptor mutants.

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First Hit

L1: Entry 1 of 6 File: PGPB Apr 29, 2004

DOCUMENT-IDENTIFIER: US 20040082510 A1
TITLE: Pyk2 phosphorylation by her3 induces tumor invasion

Summary of Invention Paragraph:
[0016] Further especially preferred is a PKY2 protein comprising:

DOCUMENT-IDENTIFIER: US 20030119067 A1
TITLE: PYK2 related products and methods

Detail Description Paragraph:
[0221] The effects of coexpression of PYK2 and Csk on PYK2 tyrosine phosphorylation and MAP kinase activation were also assessed. pRK5, PYK2, PYK2-Y402F or PYK2 plus increasing concentrations of Csk were transiently transfected in 293T cells. Total cell lysates were analyzed by immunoblotting with antibodies against phosphotyrosine, PYK2 and Csk. A weak tyrosine phosphorylation of PYK2 Y402F was observed upon longer exposure. The same lysates were used to determine MAP kinase activation. This experiment was repeated three times. It was thus determined that PKY2 and Src are involved in MAP kinas activation.

DOCUMENT-IDENTIFIER: US 6472218 B1
TITLE: Systems and methods for rapidly identifying useful chemicals in liquid samples

CLAIMS:

1. A method of modulating the activity of a selected molecular targets with a selected chemical compound, comprising: storing a set of chemical compounds in addressable wells in a chemical library; programming a chemical storage and retrieval module containing said chemical compounds for the selection of a subset of said chemical compounds; removing said subset of said addressable chemical wells from sad chemical storage module with an automated robotic retriever; routing said selected subset of addressable chemical wells away from said chemical storage and retrieval module and toward a liquid processing apparatus in a transport pathway; retrieving said programmed subset of said chemical compounds with said liquid processing apparatus by retrieving liquid samples from less than all of said removed subset of addressable chemical wells; testing, in said liquid processing apparatus, said selected set of chemical compounds for modulating activity against one or more selected molecular targets; selecting a chemical compound from among said selected set of chemical compounds that modulates that activity of at least one or more selected molecular targets; and contacting said selected chemical compound with said selected molecular target such that the activity of said selected molecular target is modulated.

3. The method of claim 1, wherein said chemical compound has modulating activity with respect to said molecular target selected from the group consisting of ion channels, N-methyl-D-aspartate receptor, kainite receptors, AMPA receptor, GABA receptors, nicotinic acetylcholine receptors, excitatory amino acid receptors, soluble proteins, membrane proteins, hormone receptors, transcription factors, proteases, kinases, and phosphatases.

4. The method of claim 1, wherein said testing comprises testing at a rate of at least 50,000 chemical compounds in 24 hours.

5. The method of claim 1, wherein said testing comprises testing at a rate of at least 100,000 chemical compounds in 24 hours.

6. The method of claim 1, wherein said testing comprises testing at a rate of at least 300,000 compounds in 24 hours.

7. The method of claim 1, wherein said testing comprises testing at a rate of at least 500,000 chemical compounds in 24 hours.

8. The method of claim 1, wherein said testing comprises testing at a rate of at least 1,000,000 chemical compounds in 24 hours.

9. The method of claim 1, wherein said testing comprises a cell based assay.

PAT-NO: 6440681
DOCUMENT-IDENTIFIER: US 6440681 B1

TITLE: Methods for identifying agonists and antagonists for human neuronal nicotinic acetylcholine receptors

DATE-ISSUED: August 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elliott; Kathryn J.	San Diego	CA		
Ellis; Steven B.	San Diego	CA		
Harpold; Michael M.	El Cajon	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Merck & Co., Inc.	Rahway	NJ			02

APPL-NO: 08/ 487596 [PALM]
DATE FILED: June 7, 1995

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation-in-part of U.S. application Ser. No. 08/149,503, filed Nov. 8, 1993, now abandoned; a continuation-in-part of U.S. application Ser. No. 08/028,031, filed Mar. 8, 1993, now abandoned; and a continuation-in-part of U.S. application Ser. No. 07/938,154, filed Nov. 30, 1992, now U.S. Pat. No. 5,981,193, which is a continuation-in-part of U.S. application Ser. No. 07/504,455, filed Apr. 3, 1990, now issued as U.S. Pat. No. 5,369,028, each of which is hereby incorporated by reference herein in their entirety.

INT-CL: [07] C12 N 15/00

US-CL-ISSUED: 435/7.2; 435/69.1, 435/252.3, 435/320.1, 435/325, 435/70.1, 435/71.1, 536/23.5
US-CL-CURRENT: 435/7.2; 435/252.3, 435/320.1, 435/325, 435/69.1, 435/70.1, 435/71.1, 536/23.5

FIELD-OF-SEARCH: 435/69.1, 435/70.1, 435/240.2, 435/252.3, 435/320.1, 435/71.1, 435/7.2, 536/23.5, 935/70

PRIOR-ART-DISCLOSED:

Entry 4 of 6 File: USPT Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6300473 B1
**** See image for Certificate of Correction ****
TITLE: SLM-1: a novel Sam68-like mammalian protein

CLAIMS:

1. An isolated polypeptide comprising at least 25 consecutive amino acids of SEQ ID NO. 1, wherein the polypeptide contains at least 2 proline motifs, can act as a substrate for a tyrosine kinase, and can act as a substrate for arginine methyltransferase.

3. A polypeptide encoded by a DNA comprising the nucleic acid sequence of SEQ ID NO. 3 wherein the polypeptide contains at least two proline motifs, can act as a substrate for a tyrosine kinase, and can act as a substrate for an arginine methyltransferase.

L13: Entry 6 of 6 File: USPT Nov 17, 1998

US-PAT-NO: 5837815
DOCUMENT-IDENTIFIER: US 5837815 A

TITLE: PYK2 related polypeptide products

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lev; Sima	San Francisco	CA		
Schlessinger; Joseph	New York	NY		

US-CL-CURRENT: 530/350; 435/69.1, 530/412

CLAIMS:

What is claimed is:

1. An isolated or purified proline-rich tyrosine kinase 2 (PYK2) polypeptide having a phosphorylation activity, said polypeptide comprising at least thirty-five contiguous amino acids of the amino acid sequence of SEQ ID NO: 1.

2. The polypeptide of claim 1, wherein said polypeptide is isolated.

3. The polypeptide of claim 1, wherein said polypeptide is purified.

4. The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:1.

5. The polypeptide of claim 4, wherein said polypeptide consists of the amino acid sequence of SEQ ID NO: 1.

6. The polypeptide of claim 1, wherein said polypeptide is able to regulate a potassium channel.

7. The polypeptide of claim 6, wherein said potassium channel is a delayed rectifier-type potassium channel.

8. The polypeptide of claim 1, wherein said polypeptide is recombinantly produced.

US005837815A

United States Patent [19]

Lev et al.

[11] Patent Number: 5,837,815

[45] Date of Patent: Nov. 17, 1998

[54] **PYK2 RELATED POLYPEPTIDE PRODUCTS**

[75] Inventors: **Sima Lev**, San Francisco, Calif.; **Joseph Schlessinger**, New York, N.Y.

[73] Assignee: **Sugen, Inc.**, Redwood City, Calif.

[21] Appl. No.: **460,626**

[22] Filed: **Jun. 2, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 357,642, Dec. 15, 1994.

[51] **Int. Cl.**[6] **C12P 12/06**; C07K 14/00; A23J 1/00

[52] **U.S. Cl.** **530/350**; 530/412; 435/69.1

[58] **Field of Search** 530/350, 412; 435/69.1

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

0520722	6/1992	European Pat. Off. .
0562734	3/1993	European Pat. Off. .
9323569	11/1993	WIPO .

OTHER PUBLICATIONS

Ezoe, K et al. Onougene 9(3): 935–8, 1994.

Johnson, JD. PMAS, USA, 90 (12): 5677–81, 1993.

Kubo et al. Nature, 323: 411–416, 1986.

Whitney et al (DNA Cell Biol., 1993, 12: 823–830).

Sambrook et al (Molecular Cloning, A Lab Manual, 1989. Cold Spring Harbor, Cold Sring Harbor Press pp. 9.5–9.51.

Aaronson, S., "Growth Factors and Cancer," *Science* 254:1146–1153 (1991).

Adelman et al., "In Vitro Deletional Mutagenesis for Bacterial Production of the 20,000–Dalton Form of Human Pituitary Growth Hormone," *DNA* 2(3):183–193 (1983).

Allen et al., "Modulation of CD4 by suramin" *Clin. Exp. Immunol.* 91:141–146 (1991).

Anafi et al., "Tyrphostin–Induced Inhibition of p120$^{bcr-abl}$ Tyrosine Kinase Activity Induces K562 to Differentiate," *Blood* 82:3524–3529 (1993).

Baker et al., "Induction of acetylcholine receptor clustering by native polystyrene beads" *Journal of Cell Science* 102:543–555 (1992).

Barker et al., "In vitro activity of non–glutamate containing quinazoline–based thymidylate synthase inhibitors" *Proceedings of the American Association for Cancer Research* 32:327 (#1939) (1991).

Benoist and Chambon, "In vivo sequence requirements of the SV40 early promoter region," *Nature* 290:304–310 (1981).

Bertino, "Toward Improved Selectivity in Cancer Chemotherapy: The Richard and Hinda Rosenthal Foundation Award Lecture" *Cancer Research* 39:293–304 (Feb. 1979).

Bilder et al., "Tyrphostins inhibit PDGF–induced DNA synthesis and associated early events in smooth muscle cells," *Am. I. Physiol.* 260 (Cell Physiol.29):C721–C730 (1991).

Bird et al., "Single–Chain Antigen–Binding Proteins," *Science* 242:423–426 (1988).

Bitter et al., "Expression and Secretion Vectors for Yeast," *Methods in Enzym.* 153:516–544 (1987).

Brinster et al., "Factors Affecting the Efficiency of Introducing Foreign DNA into Mice by Microinjecting Eggs," *Proc. Natl. Acad. Sci. USA* 82:4438 (1985).

Brown and Bimbaumer, "Ionic Channels and Their Regulation by G Protein Subunits," *Ann. Rev. Physiol.* 52:197–213 (1990).

Brunton et al., "Anti–tumour activity of novel tryphostins in breast cancer cells." *Proceedings of the American Association for Cancer Research* 33:558 (1992) (#3335).

Bryckaert et al., "Inhibition of Platelet–Derived Growth Factor–Induced Mitogenesis and Tyrosirie Kinase Activity in Cultured Bone Marrow Fibroblasts by Tyrphostins," *Exp. Cell Research* 199:255–261 (1992).

Burke Jr. et al., Bicyclic Compounds as Ring–Constrained Inhibitors of Protein–Tyrosine Kinase p56^{Lck} [1] *Journal of Medicinal Chemistry* 36(4):425–432 (1993).

Burke et al., "Arylamides of Hydroxylated Isoquinolines as Protein–Tyrosine Kinase Inhibitors" *Bioorganic & Medical Chemistry Letters* 2(12):1771–1774 (1992).

Cantrell, D., "G proteins in lymphocyte signalling," *Current in Immunology* 6:380–384 (1994).

Carmeliet, "Ion channel agonists: expectations for therapy," *Eur. Heart. J.* 12:30–37 (1991).

Chen and Okayama, "High–Efficiency Transformation of Mammalian Cells by Plasmid DNA," *Mol. and Cell. Biol.* 7(8):2745–2752 (1987).

Chowdhury et al., "Long–term Improvement of Hypercholesterolemia After Ex Vivo Gene Therapy in LDLR–Deficient Rabbits," *Science* 254:1082 (1991).

Cobb et al., "Stable Association of pp60^{src} and pp59^{fyn} with the Focal Adhesion–Associated Protein Tyrosine Kinase, pp 125^{FAK}," *Mol. and Cell. Biol.* 14:147–155 (1994).

Colbère–Garapin et al., "A New Dominant Hybrid Selective Marker for Higher Eukaryotic Cells," *J. Mol. Biol.* 150:1–14 (1981).

Cole et al., "The EBV–Hybridoma Technique and its Application to Human Lung Cancer," pp. 77–96 in *Monoclonal Antibodies and Cancer Therapy* eds. Reisfeld and Sell, Alan R. Liss, Inc., New York (1985).

Creighton, T., *Proteins: Structures and Molecular Principles* pp. 79–86, W.H. Freeman and Co., New York, (1983).

Cristiano et al., "Hepatic Gene Therapy: Adenovirus Enhancement of Receptor–Mediated Gene Delivery and Expression in Primary Hepatocytes," *Proc. Natl. Acad. Sci. USA* 90:2122–2126 (1993).

(List continued on next page.)

Primary Examiner—Lila Feisee
Assistant Examiner—Susan Ungar
Attorney, Agent, or Firm—Lyon & Lyon LLP

[57] **ABSTRACT**

The present invention features a method for treatment of an organism having a disease or condition characterized by an abnormality in a signal transduction pathway, wherein the signal transduction pathway include a PYK2 protein. The invention also features methods for diagnosing such diseases and for screening for agents that will be useful in treating such diseases. The invention also features purified and/or isolated nucleic acid encoding a PYK2 protein.

8 Claims, 4 Drawing Sheets

L25: Entry 2 of 33 File: USPT Oct 5, 2004

US-PAT-NO: 6800447
DOCUMENT-IDENTIFIER: US 6800447 B2

TITLE: Methods for identifying compounds which bind the active CCR5 chemokine receptor

DATE-ISSUED: October 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Samson; Michel	Gentilly			FR
Parmentier; Marc	Linkebeek			BE
Vassart; Gilbert	Brussels			BE
Libert; Frederick	Braine-L'Alleud			BE

US-CL-CURRENT: 435/7.1; 435/6, 435/69.1, 435/7.21, 435/70.1, 435/70.3, 436/501, 530/350, 530/387.9

CLAIMS:

We claim:

1. A method of identifying a compound which binds to a polypeptide sequence comprising one of SEQ ID NO: 5, comprising contacting said polypeptide with a candidate compound and detecting binding of said candidate compound to said polypeptide.

2. A method for identifying a compound which specifically binds to the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO: 5, the method comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor; (b) expressing said receptor under conditions permitting specific binding of said compound to said receptor; (c) exposing said cell to said compound; and (d) detecting the presence of said compound which has specifically bound to said receptor, thereby determining whether said compound specifically binds to said receptor.

3. The method according to claim 2, wherein said detecting is performed by monitoring a change in the G- protein coupled signaling activity of said CCR5 chemokine receptor.

4. The method according to claim 3, wherein said detecting is performed by monitoring the level of inositol triphosphate.

5. The method according to claim 3, wherein said detecting is performed by monitoring the level of intracellular calcium in said host cell.

6. The method according to claim 2, wherein said detecting is performed by

measuring the modifications of cell metabolism resulting from the stimulation of an intracellular cascade.

7. The method according to claim 6, wherein said modifications of cell metabolism are detected by monitoring the acidification rate of said host cell. cell.

8. The method of claim 2, further comprising measuring the infectivity of the cell from said step (c) by HIV in the presence of the detected compound from step (d), wherein a decrease in HIV infectivity of said cell from said step (c) relative to that of said cell from said step (b) which was not exposed to said compound, indicates that said compound inhibits the ability of HIV-1 to utilize said CCR5 chemokine receptor as a cofactor.

9. The method according to claim 8, wherein said infectivity of the cell by HIV HIV is measured by measuring the production of an HIV protein.

10. The method according to claim 9, wherein said HIV protein is p24.

11. The method of claim 8, wherein said compound decreases infectivity by HIV by at least two-fold.

12. A method for identifying a compound which specifically binds to the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO: 5, the method comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor. (b) expressing said receptor by said cell. (c) preparing a cell extract from the cell transfected with said nucleic acid molecule, (d) isolating a membrane fraction of said cell extract, (e) contacting said compound with said membrane fraction under conditions permitting binding of the compound to said fraction, and (f) detecting the presence of said compound which has specifically bound to said receptor, wherein said detection indicates that said compound specifically binds to said receptor.

13. A method for identifying a compound as an agonist of the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO:5, comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor, (b) expressing said receptor under conditions Permitting specific binding of said compound to said receptor; (c) contacting the cells from part (b) with said compound under conditions permitting the activation of a functional peptide response from the cell, and (d) detecting said response,

wherein the detection of an increase in said response indicates that the compound is an agonist of said CCR5 chemokine receptor.

14. A method for identifying a compound as an antagonist of the CCR5 chemokine receptor whose amino acid sequence is SEQ ID NO:5, comprising the steps of: (a) transfecting a cell with a nucleic acid molecule encoding said receptor, (b) expressing said receptor in the transfected cells of part (a), (c) contacting the cells from part (b) with said compound in the presence of an agonist of said receptor, under conditions permitting the activation of a functional response from the cell, and d) detecting said response,

wherein the detection of a decrease in said response relative to the response detected from contacting the cells from part (b) in the presence of said agonist but in the absence of said compound indicates that the compound is an antagonist of said CCR5 chemokine receptor.

15. The method according to any one of claim 2, 12, 13, or 14, wherein said cell is selected from the group consisting of CHO-K1, HEK293, BHK21, and COS-7. 7.

16. The method according to claim 14, wherein said agonist for the CCR5 receptor is the CCR5 chemokine.

17. The method according to claim 14, wherein said agonist is the CCR5 chemokine, and wherein said CCR5 chemokine is labeled.

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L21: Entry 66 of 67 File: USPT Jun 9, 1998

US-PAT-NO: 5763198
DOCUMENT-IDENTIFIER: US 5763198 A

TITLE: Screening assays for compounds

DATE-ISSUED: June 9, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hirth; Klaus Peter	San Francisco	CA		
App; Harald	San Francisco	CA		
Tsai; Jianming	San Francisco	CA		

US-CL-CURRENT: 435/7.21; 435/15, 435/21, 435/29, 435/7.23, 435/7.24, 435/7.94, 436/518, 436/548

CLAIMS:

What is claimed is:

1. An assay for screening test substances for the ability to modulate activity of a specific protein tyrosine kinase involved in signal transduction, comprising:

(a) contacting (i) a lysate of a target cell which was exposed to the test substance with (ii) an anchoring molecule specific for a protein substrate which is phosphorylated as a result of signal transduction in the target cell, under conditions and for a time sufficient to allow binding of the protein substrate to the anchoring molecule thereby rendering the protein substrate immobilized; and

(b) detecting phosphotyrosine residues on any protein substrate bound to the anchoring molecule, in which differences in the detection of phosphotyrosine residues on the immobilized protein substrate derived from lysates of the target cells which were exposed to the test substance as compared to that of immobilized protein substrate derived from control target cells which were not exposed to the test substance, indicate that the test substance modulates the activity of the tryrosine kinase involved in a signal transduction.

2. The assay of claim 1 in which the anchoring molecule is a antibody.

3. The assay of claim 1 in which the phosphotyrosine residue is detected using an antibody specific for phosphotyrosine.

4. The assay of claim 3 in which the antibody specific for phosphotyrosine is a monoclonal antibody.

5. The assay of claim 3 in which the antibody specific for phosphotyrosine is labeled.

6. The assay of claim 5 in which the label is selected from the group

consisting of a radiolabel, a fluorescent label, a luminescent label and an enzymatic label.

7. The assay of claim 3 in which the antibody specific for phosphotyrosine is indirectly labeled.

8. The assay of claim 7 in which the antibody specific for phosphotyrosine is indirectly labeled with a label selected from the group consisting of a radiolabeled anti-immunoglobulin, a fluorescently labeled anti-immunoglobulin, a luminescently labeled anti-immunoglobulin, and an enzymatically labeled anti anti-immunoglobulin.

9. The assay of claim 2 in which the antibody is bound to a microtiter plate.

10. An assay for screening test substances for the ability to modulate activity activity of a specific protein tyrosine phosphatase involved in signal transduction, comprising:

(a) contacting (i) a lysate of a target cell which was exposed to the test substance with ~~(ii) an immobilized~~ antibody specific for a protein substrate which is dephosphorylated as a result of signal transduction in the target cell, under conditions and for a time sufficient to allow binding of the protein substrate to the immobilized antibody thereby rendering the protein substrate immobilized; and

(b) detecting phosphotyrosine residues on any protein substrate bound to the immobilized antibody, in which differences in the detection of phosphotyrosine residues on the immobilized protein substrate derived from lysates of the target cells which were exposed to the test substance as compared to that of immobilized protein substrate derived from control target cells which were not exposed to the test substance, indicate that the test substance modulates the activity of the tyrosine phosphatase involved in a signal transduction.

11. The assay of claim 10 in which the anchoring molecule is a antibody.

12. The assay of claim 10 in which the phosphotyrosine residue is detected using an antibody specific for phosphotyrosine.

13. The assay of claim 12 in which the antibody specific for phosphotyrosine is a monoclonal antibody.

14. The assay of claim 12 in which the antibody specific for phosphotyrosine is labeled.

15. The assay of claim 14 in which the label is selected from the group consisting of a radiolabel, a fluorescent label, a luminescent label and an enzymatic label.

16. The assay of claim 12 in which the antibody specific for phosphotyrosine is indirectly labeled.

17. The assay of claim 16 in which the antibody specific for phosphotyrosine is indirectly labeled with a label selected from the group consisting of a radiolabeled anti-immunoglobulin, a fluorescently labeled anti-immunoglobulin, a luminescently labeled anti-immunoglobulin, and an enzymatically labeled anti-immunoglobulin.

18. The assay of claim 11 in which the antibody is bound to a microtiter plate. plate.

19. The assay of claim 1 or claim 10, wherein the test substance is a therapeutic compound that modulates kinase or phosphatase activity and the assay is used to determine whether said target cell will respond to said therapeutic compound.

20. The assay of claim 19, in which the target cell is a cell in which a pathological process correlating with a disease state is derived from a patient patient exhibiting said disease state, thereby determining whether said target cell will respond to said therapeutic compound.

21. The assay of claim 20, in which the disease state is selected from the group consisting of neoplasia, cancer and diabetes.

22. An assay for screening test substances for the ability to modulate the level of phosphotyrosine on a specific protein substrate involved in signal transduction, comprising:

(a) contacting a lysate of a target cell, after the target cell was exposed to a test substance, with an immobilized antibody specific for a protein substrate which is phosphorylated or dephosphorylated as a result of signal transduction in the target cell, under conditions and for a time sufficient to allow binding of the protein substrate to the immobilized antibody thereby rendering the protein substrate immobilized; and

(b) detecting phosphotyrosine residues on any protein substrate bound to the immobilized antibody, in which differences in the detection of phosphotyrosine residues on the immobilized protein substrate derived from lysates of the target cells which were exposed to the test substance as compared to that of immobilized protein substrate derived from control target cells which were not exposed to the test substance, indicate that the test substance modulates the phosphotyrosine lever of the protein substrate.

US005763198A

United States Patent [19]

Hirth et al.

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[54] SCREENING ASSAYS FOR COMPOUNDS

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[58] Field of Search 435/7.21, 7.23, 435/7.24, 7.4, 7.94, 15, 21, 29; 436/518, 548

[56] References Cited

U.S. PATENT DOCUMENTS

4,215,051	7/1980	Schroeder et al.	260/346.7
4,478,946	10/1984	Van der Merwe et al.	436/518
4,946,778	8/1990	Ladner et al.	435/69.6
5,427,916	6/1995	Gerwitz et al.	436/813
5,527,688	6/1996	Millia	435/15
5,538,858	7/1996	Mallia	435/15
5,580,742	12/1996	Bodenmuller et al.	435/7.94
5,599,681	2/1997	Epstein et al.	435/7.23

FOREIGN PATENT DOCUMENTS

WO 90/10234	9/1990	WIPO .
WO 93/21230	10/1993	WIPO .

OTHER PUBLICATIONS

Millauer et al., 1994, "Glioblastoma Growth Inhibited in vivo by a Dominant-Negative Flk-1 Mutant," Nature 367:576–579.

Fu et al., 1993, "Transcription Factor p91 Interacts with the Epidermal Growth Factor Receptor and Mediates Activation of the c-fos Gene Promoter," Cell 74:1135–1145.

Musacchio et al., 1993, "The PH Domain: a Common Piece in the Structural Patchwork of Signalling Proteins," TBIS 18:343–348.

Katagiri et al., 1993, "Tyrosine-Phosphorylation of Tubulin during Nonocytic Differentiation of HL-60 Cells," J. Immunol. 150:585–593.

Schraag et al., 1993, "Standardization of an Enzyme-Linked Immunosorbent Assay for the Determination of Protein Tyrosine Kinase Activity," Analytical Biochem. 211:233–239.

Walton et al., 1993, "Protein Tyrosine Phosphatases," Annu. Rev. Biochem. 62:101–20.

Pawson et al., 1993, "SH2 and SH3 Domains," Current Biology 3:434–442.

Hansen et al., 1993, "Application of Two-Dimensional Gel Analysis to Identification and Characterization of Tyrosine Phosphorylated Substrates for Growth Factors Receptors," Electrophoresis 14:112–126.

Campbell et al., 1993, "Evidence for Involvement of the Growth Hormone Receptor-Associated Tyrosine Kinase in Actions of Growth Hormone," J. Biol. Chem. 268:7427–7434.

Pawson et al., 1992, "SH2 and SH3 Domains: From Structure to Function," Cell 71:359–362.

Schlessinger et al., 1992, "Growth Factor Signalling by Receptor Tyrosine Kinases," Neuron 9:383–391.

Pelicci et al., 1992, "A Novel Transforming Protein (SHC) with an SH2 Domain is Implicated in Mitogenic Signal Transduction," Cell 70:93–104.

Farley et al., 1992, "Development of Solid-Phase Enzyme-Linked Immunosorbent Assays for the Determination of Epidermal Growth Factor Receptor and pp60$^{c\text{-}src}$ Tyrosine Protein Kinase Activity," Analytical Biochem. 203:151–157.

Peraldi et al., 1992, "Dephosphorylation of Human Insulin-Like Growth Factor I (IGF-I) Receptors by Membrane-Associated Tyrosine Phosphatases," Biochem J. 285:71–78.

Koch et al., 1991, "SH2 and SH3 Domains: Elements That Control Interactions of Cytoplasmic Signaling Proteins," Science 252:668–674.

Donato et al., 1991, "Tumor Necrosis Factor Regulates Tyrosine Phosphorylation on Epidermal Growth Factor Receptors in A431 Carcinoma Cells: Evidence for a Distinct Mechanism," Cell Growth & Differentiation 3:259–268.

Aaronson et al., 1991, "Growth Factors and Cancer," Science 254:1146–1153.

Ueno et al., 1991, "Inhibition of PDGF β Receptor Signal Transduction by Coexpression of a Truncated Receptor," Science 252:844–848.

Skolinik et al., 1991, "Cloning of P13 Kinase-Associated p85 Utilizing a Novel Method for Expression/Cloning of Target Proteins for Receptor Tyrosine Kinases," Cell 65:83–90.

Cantley et al., 1991, "Oncogenes and Signal Transduction," Cell 64:281–302.

Lazaro et al., 1991, "Description of an Enzyme-Linked Immunosorbent Assay for the Detection of Protein Tyrosine Kinase," Analytical Biochem. 192:257–261.

Tonks et al., 1991, "Purification of Protein-Tyrosine Phosphatases from Human Placenta," Methods in Enzymology 201:427–442.

(List continued on next page.)

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[57] ABSTRACT

The invention is directed to rapid and quantitative assay systems for screening test compounds for their ability to modulate tyrosine kinase or phosphatase activities involved in signal transduction by determining the tyrosine phosphorylation state of a protein substrate using an anti-phosphotyrosine antibody and an antibody specific for the protein substrate. These assays may be practiced in a whole cell or cell-free system. The assays can be used to identify test compounds for use in therapeutic applications to disease processes in which tyrosine kinase or phosphatase activity in a signal transduction pathway contributes to a pathological process.

22 Claims, No Drawings

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DOCUMENT-IDENTIFIER: US 5880141 A

TITLE: Benzylidene-Z-indoline compounds for the treatment of disease

DATE-ISSUED: March 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 514/339; 514/414, 514/418, 546/277.4, 548/468, 548/486

CLAIMS:

What is claimed:

1. A method for treating diseases related to tyrosine kinase signal transduction comprising administering to a subject in need of such treatment an an effective amount of a pharmaceutical composition, wherein the composition comprises a compound selected from the group consisting of 3-benzylidene-2-indolinone; 3-(2,5-dimethoxybenzylidene)-2-indolinone; 3-[(pyrid-4-yl) methylene]-2-indolinone; 3-(4-nitrobenzylidene)-2-indolinone; and 3-(4-trifluoromethylbenzylidene)-2-indolinone in combination with a pharmaceutically pharmaceutically acceptable carrier or excipient.

2. A method for regulating, modulating or inhibiting tyrosine kinase signal transduction comprising administering to a subject in need of such treatment an an effective amount of a compound selected from the group consisting of 3-benzylidene-2-indolinone; 3-(2,5-dimethoxybenzylidene)-2-indolinone; 3-[(pyrid-4-yl)methylene]-2-indolinone; 3-(4-nitrobenzylidene)-2-indolinone; and 3-(4-trifluoromethylbenzylidene)-2-indolinone in combination with a pharmaceutically acceptable carrier or excipient.

3. The method of claim 1 wherein said disease is selected from the group consisting of: cancer, blood vessel proliferative disorders, fibrotic disorders, mesangial cell proliferative disorders and metabolic diseases.

4. The method of claim 3 wherein the blood vessel proliferative disorder is selected from the group consisting of arthritis and restenosis.

5. The method of claim 3 wherein the fibrotic disorder is selected from the group consisting of hepatic cirrhosis and atherosclerosis.

6. The method of claim 3 wherein the mesangial cell proliferative disorder is selected from the group consisting of glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, transplant rejection and glomerulopathies.

7. The method of claim 3 wherein the metabolic disorder is selected from the group consisting of psoriasis, diabetes mellitus, wound healing, inflammation

and neurodegenerative diseases.

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United States Patent [19]

Tang et al.

[11] Patent Number: 5,880,141

[45] Date of Patent: Mar. 9, 1999

[54] **BENZYLIDENE-Z-INDOLINE COMPOUNDS FOR THE TREATMENT OF DISEASE**

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[73] Assignee: **Sugen, Inc.**, Redwood City, Calif.

[21] Appl. No.: **485,323**

[22] Filed: **Jun. 7, 1995**

[51] **Int. Cl.**[6] **A61K 31/405**; C07D 209/34

[52] **U.S. Cl.** **514/339**; 514/414; 514/418; 546/273; 548/468; 548/486

[58] **Field of Search** 514/339, 414, 514/418; 548/468, 486; 546/273

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,872,372	2/1959	Hull	514/418
2,968,557	1/1961	Burgardt et al.	514/269
4,642,309	2/1987	Michel et al.	514/269
4,826,847	5/1989	Michel et al.	514/256
4,853,403	8/1989	Shiraishi et al.	514/404
4,868,304	9/1989	Larock et al.	546/141
4,966,849	10/1990	Vallee et al.	435/199
4,971,996	11/1990	Shiraishi et al.	514/521
5,051,417	9/1991	Nadler et al.	514/222.5
5,057,538	10/1991	Shiraishi et al.	514/521
5,089,516	2/1992	Shiraishi et al.	514/404
5,124,347	6/1992	Conner et al.	514/418
5,202,341	4/1993	Shiraishi et al.	514/369
5,206,261	4/1993	Kawaguchi et al.	514/418
5,217,999	6/1993	Levitzki et al.	514/613
5,302,606	4/1994	Spada et al.	514/357
5,322,950	6/1994	Sircar et al.	548/253
5,330,992	7/1994	Eissenstat et al.	514/312
5,374,652	12/1994	Buzzetti et al.	514/418
5,382,593	1/1995	Le Baut et al.	514/418
5,397,787	3/1995	Buzzetti et al.	514/300
5,409,949	4/1995	Buzzetti et al.	514/414
5,463,052	10/1995	Haga et al.	544/2

FOREIGN PATENT DOCUMENTS

286870	11/1967	Australia .
2012634	9/1991	Canada .
351213	1/1990	European Pat. Off. .
525472	2/1993	European Pat. Off. .
566 266	10/1993	European Pat. Off. .
580502	1/1994	European Pat. Off. .
0626377	11/1994	European Pat. Off. .
632102	1/1995	European Pat. Off. .
0 632 102	4/1995	European Pat. Off. .
662473	7/1995	European Pat. Off. .
0788890	8/1997	European Pat. Off. .
1.398.224	3/1965	France .
1398224	5/1965	France .
2689397	8/1993	France .
878539	6/1953	Germany .
2159360	4/1973	Germany .
2159361	4/1973	Germany .
2159362	4/1973	Germany .
2159363	4/1973	Germany .
2321656	11/1973	Germany .
3310891	9/1984	Germany .
3426419	1/1986	Germany .
3899/92-		
65452	10/1992	Hungary .
62-29570	2/1987	Japan .
62-39564	2/1987	Japan .
63-141955	6/1988	Japan .
5-58894	3/1993	Japan .
9286777	11/1997	Japan .
809691	3/1959	United Kingdom .
835473	5/1960	United Kingdom .
1384599	2/1975	United Kingdom .
WO 88/07035	9/1988	WIPO .
WO 91/13055	5/1991	WIPO .

(List continued on next page.)

OTHER PUBLICATIONS

Beilstein Reg. No.: 252929 Beilstein (1923).

Translation of Simonnot et al., "Process for Dyeing Textile Materials Made From Polyacrylonitrile" *Imperial Chemical Industries Ltd.* 1–8 Patent Specification (1965).

Translation of Neber et al., "On the action of Benzaldehydes on the Free Aminophenylacetic Acid (II)" *Chem. Institute of the Univ. of Tubingen* 1–8 (1923).

Translation of Szanatay et al., "Process for Preparing Spiro–oxindole Derivatives and of Drug Preparation Containing Same" 1–29 (1992).

Andreani et al., "Potential Antitumor Agents. 25[1]. Synthesis and Cytotoxic Activity of 3–(2–Chloro–3–Indolylmethlene) 1,3–Dihydroindol–2–Ones," *AntiCancer Research* 16:3585–3588 (1996).

Terrett et al., "Combinatorial Synthesis—The Design of Compound Libraries and their Application to Drug Discovery," Tetrahedron 51(30):8135–8173 (1995).

Coda et al., "3–(4–methylbenzilidene)–1–, 3–dihydroindol–2–one," *Journal of the Chemical Society, Perkin Transactions 2* 4:615–620 (1984), Database Crossfire, Beilstein No. 6–21.

Wahl, "3–benzilidene–5–methyl–1,3–dihydroindol–2–one," *Ann. Chim* p. 350 (1926), Database Crossfire, Beilstein No. 2 21–00–00290.

Elliot, "1–methyl–2–(3–oxindolidenmethyl)–pyridinium," *Journal of Organic Chemistry* 29:2438–2440 (1964), Database Crossfire, Beilstein No. 5–24.

Howard, Provisional Patent Application No. 60/015,134 filed Mar. 29, 1996 for "Lactam Derivatives".

Abramovitch, R. A., and Hey, D. H. (1954). Internuclear cyclisation. J. Chem. Soc., 1697–1703.

Abramovitch, R. A., Coutts, R. T., and Pound, N. J. (1967). A novel synthesis of a cyclic hydroxamic acid involving a molecular rearrangement. Chemistry and Industry 44, 1871.

(List continued on next page.)

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[57] **ABSTRACT**

The present invention relates to organic molecules capable of modulating tyrosine kinase signal transduction in order to regulate, modulate and/or inhibit abnormal cell proliferation.

7 Claims, No Drawings